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Structure Elucidation and Synthesis of Natural Products

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by

Annabel Christine Murphy

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Abstract

In this thesis, synthetic chemistry was used as a tool in the exploration of various aspects of natural products discovered by the natural products research group at the University of Canterbury.

Work on the constituent amino acids and connectivity of the pteratides, a potently cytotoxic series of cyclodepsipeptides, had been completed before the beginning of this work (carried out by Miss C. Chen). The elucidation of the stereochemistry of the constituent amino acids was undertaken in this present work. The synthesis of all stereochemical entities of a number of unusual amino acids, which were either not available commercially or were expensive, was carried out, providing reference materials for comparison to the natural products. The synthesis of the diastereoisomers of one of these amino acids, 4-methylproline, was carried out by modification of literature procedures, which led to the development of an improved, concise and stereoselective synthesis. The hydrolysis of the natural products, derivatisation of the resultant hydrolysates, synthetic and commercial reference amino acids and HPLC analysis allowed the full stereochemical assignment of the pteratide series.

The total synthesis of *spiro*-mamakone A, a cytotoxic polyketide isolated by Dr S. van der Sar, was undertaken. The synthesis was not successfully completed due to difficulties in the late-stage formation of a crucial enedione motif. However, very advanced intermediates were successfully synthesised. These synthetic analogues of the natural product were analysed for biological activity, allowing valuable insight into the structure-activity relationship, for example, demonstrating the importance of the enedione moiety to biological activity.

Table of Contents

Abbreviations

1D	one dimensional
2D	two dimensional
9-BBN	9-Borabicyclo[3.3.1]nonane
Ac_2O	acetic anhydride
AIBN	azobisisobutyronitrile
Boc_2O	*t*-butyl dicarbonate
br s	broad singlet (in NMR)
c	concentration (in optical rotation)
C_{18}	octadecyl phase (chromatography packing phase)
calcd	calculated
CBz	benzyloxycarbonyl
CBzCl	benzyloxycarbonyl chloride
CIGAR	constant time inverse-detection gradient accordion rescaled (in NMR)
CIMS	chemical ionisation mass spectrometry
COSY	correlation spectroscopy (in NMR)
d	doublet (in NMR)
δ	chemical shift in parts per million
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	dichloromethane
dd	doublet of doublets
DDQ	dichlorodicyanoquinone
DEAD	diethyl azodicarboxylate
DHN	1,8-dihydroxynaphthalene
DIBAL-H	diisobutylaluminium hydride
DMAP	dimethylaminopyridine
DMF	dimethylformamide
DMP	Dess-Martin periodinane / 3,5-dimethylpyrazole
DMSO	dimethylsulfoxide

dt	doublet of triplets
DTS	diverted total synthesis
EIMS	electron ionisation mass spectrometry
ESIMS	electrospray ionisation mass spectrometry
ESI MS/MS	electrospray ionisation tandem mass spectrometry
eq	equivalent
Fmoc	9-fluorenylmethoxycarbonyl
FmocCl	9-fluorenylmethoxycarbonyl chloride
HPLC	high pressure liquid chromatography
HRCIMS	high resolution chemical ionisation mass spectrometry
HREIMS	high resolution electron ionisation mass spectrometry
HRESIMS	high resolution electrospray ionisation mass spectrometry
HRFABMS	high resolution fast atom bombardment ionisation mass spectrometry
HSQC	heteronuclear single quantum coherence (in NMR)
IBX	*o*-iodoxybenzoic acid
IC_{50}	concentration of sample required to inhibit cell growth by 50%
IR	infra-red
KHMDS	potassium hexamethyldisilazane
LCMS	liquid chromatography mass spectrometry
m	multiplet (in NMR)
m/*z*	mass-to-charge ratio (in mass spectrometry)
MDR	multi-drug resistance
MeCN	acetonitrile
MeOH	methanol
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MsCl	mesyl chloride
NBS	*N*-bromosuccinimide
NMO	*N*-methylmorpholine-*N*-oxide
NMR	nuclear magnetic resonance
NOESY	nuclear Overhauser enhancement spectroscopy (in NMR)
PCC	pyridinium chlorochromate

PDC	pyridinium dichromate
PMA	phosphomolybdic acid
ppm	parts per million (in NMR and errors in mass spectrometry)
pTSA	*p*-toluenesulfonic acid
q	quartet (in NMR)
R_f	retention factor
R_t	retention time
s	singlet (in NMR)
t	triplet (in NMR)
TBAF	tetra-*n*-butylammonium fluoride
TBDMSCl	*t*-butyldimethylsilyl chloride
TCCA	trichloroisocyanuric acid
TEA	triethylamine
TEMPO	2,2,6,6-Tetramethylpiperidine-1-oxyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
tRNA	transfer ribonucleic acid
TsCl	tosyl chloride
UV	ultra-violet

Chapter 1

Introduction

1.1 Natural products

The wealth of biological molecules made by nature are commonly classified into two classes, the primary and secondary metabolites. The basic metabolism and reproduction of an organism involves essential primary metabolites. However, many organisms, and in particular microorganisms, are known to produce metabolites which are non-essential, but which are believed to improve the overall 'fitness' of the producing organism. These are described as secondary metabolites, or alternatively, as natural products.

Why secondary metabolites are produced has been the subject of a great deal of debate, which continues to this day.[1,2] The role these natural products play in the producing organism is sometimes obvious, be it as a deterrent effect on would-be predators or resource competitors, or in communication, for example to attract potential mates. However, many organisms produce a vast array of secondary metabolites, many or all of which having no known role. This may be explained simply by accepting that whilst the role is not obvious to the outside observer, a role for a given metabolite may still exist, or at least have existed in the past. This view is being countered by a fascinating recent hypothesis put forward by Firn and Jones; the Screening Hypothesis.[2] This theory is based on the premise that potent biological activity is a rare property for any one molecule to possess. The authors therefore suggest that instead of carefully evolving a complex biosynthetic pathway to discreet potent metabolites, organisms endeavour to create natural product libraries, even if many of the metabolites eventually never play any role in the fitness of the organism. In this way the producer has an array of 'chemical weapons' which will be screened during interactions with other organisms, one or more of which may confer an advantage to the producer. This theory consciously draws

parallels with the high-throughput screening processes employed by man in the search for biologically useful compounds. The economics of natural selection requires that the production of secondary metabolites must not 'cost' the organism more than the benefit derived. That organisms would produce whole libraries of secondary metabolites on the off-chance one might be useful therefore runs counter to classical thinking. The authors suggest that this expense is minimised by the use of both promiscuous enzymes with broad substrate specificity and by matrix pathways, where several promiscuous enzymes act upon each other's products to generate an array of metabolites. The promiscuity of enzymes involved in secondary metabolite production is becoming increasingly accepted. The Screening Hypothesis remains contentious, however.[3,4]

Whatever the precise advantage a natural product confers to its producing organism, its ultimate raison d'être is to interact with other biological molecules. As such, man has long sought to harness nature's treasure-trove to his own advantage, with natural products being selected both historically and currently to improve his own 'fitness'.

1.2 A historical perspective

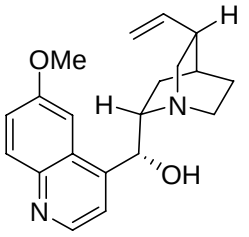
An ancient Indian legend tells of Jivaka, student of medicine with the learned guru Atreya, who, after many years of tutelage, was set the task of collecting plants which lacked any medicinal properties in the area around their ashram. After combing the area, examining leaves and flowers, and tasting fruit, he returned to Atreya to sorrowfully report that he was not able to bring back a single plant which was without any sort of medicinal value. Delighted, Atreya declared him ready to go out to help the people.[5]

The medicinal value of the natural world has been examined and exploited by almost every society for thousands of years. Some of the earliest records of ancient pharmacognosy include 660 clay tablets created around 1700 BC, documenting the medicines used in Mesopotamia. They were found in the library of the last king of Assyria, and were unwittingly preserved, by baking, by invaders who set the palace on

fire.[6] Many of these medicines were also included in the *Ebers Papyrus*, an Egyptian document dating from around 1550 BC. The similarities in pharmacognosy between these two ancient civilisations are indicative of the extent of their trading, both in goods and ideas. The medicinal knowledge of other contemporary civilisations was also documented, in the *Charaka Samhita* in India, and the *Pen Ts'ao Ching* in China, although the age of these documents is not as well defined. These cultures frequently listed plant-based remedies, although fungi also featured. The ancient Greeks are well known for their interest in medicine, with Hippocrates commonly called the 'father of medicine'. They used herbs and roots, and the herbal *De Materia Medica* by Dioscorides described over 600 plants for medicinal purposes. Many of the herbs described in these ancient texts are still in use today to treat a range of ailments.[7]

In Mesoamerica, the Aztecs and the Mayans both utilised plants and mushrooms for medicinal purposes. The Aztecs used African day flower (*Tagetes lucida*) and wormwood (*Artemisia mexicana*) to cure a range of ills including fevers and digestive problems.[8] They also used a concentrated sap of the maguey leaf (*Agave* spp.) to treat wounds due to its antibacterial properties. The Mayans treated intestinal infections with a fungus grown on roasted green corn.[9]

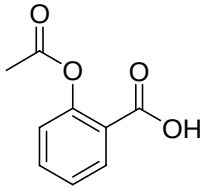
The advent of colonialism allowed the huge biodiversity of the new world to be exploited by the old world. The ipecacuanha root (*Cephalis ipecacuanha*) was introduced in Europe from South America as a treatment for amoebic dysentery.[6] Another example is cinchona bark, which was a traditional remedy amongst the indigenous peoples of Peru for fever, and which yielded one of the most celebrated natural product medicines ever discovered. In a rather unfair swap, the Europeans brought malaria, caused by several species of the parasite *Plasmodium*, to South America and obtained in return an excellent treatment for the disease; quinine from cinchona bark.[10] Quinine, **1.1**, was amongst the first drugs to be obtained in pure form by extraction from the natural source and was the first to be used therapeutically in its pure form.[6]



1.1

The massive demand for quinine caused by the prevalence of the disease, particularly in those areas being fought for by the colonising powers of the time, almost led to the extinction of the producing tree. This led to a serious problem in the supply of the drug. The issue of supply is one of the most serious problems faced by natural products as drugs and is a theme that will be discussed further later.

The utility of the bark of the willow tree (*Salix* spp.) was already known to the Greeks and the Romans for the relief of pain, and this traditional medicine lead to the development of one of the most popular analgesics and antipyretics, still widely used today, aspirin® (**1.2**).[11]



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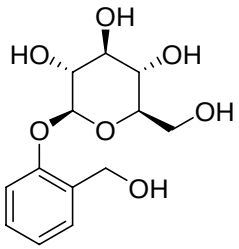

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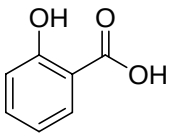


1.4

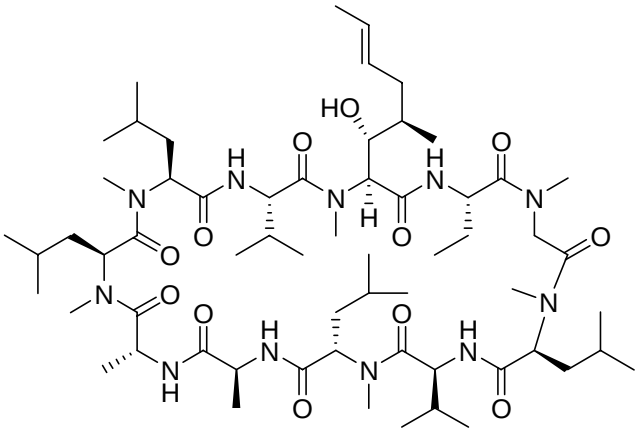
The active constituent of willow tree bark is salicin (**1.3**) which is converted *in vivo* to salicylic acid, **1.4**. The semi-synthesis of aspirin® by acetylation of salicylic acid generated a drug which was both more palatable and less irritating to the gut than either **1.3** or **1.4**. Aspirin® was the first semi-synthetic drug to be sold.

Arguably the single most important development in modern medicine was the discovery of antibiotic agents from fungal sources. In the early 1900's many farmhouses in Europe kept a loaf of mouldy rye bread, pieces of which could be mixed with water into a paste that was used to treat cuts and burns.[9] While the first report of bacterial inhibition by the

fungus *Penicillium* was in 1870 (John Burdon-Sanderson),[9] it is Alexander Fleming who is credited with the discovery and initial scientific studies of this action. He noted, in 1928, that a rare strain of *Penicillium notatum* caused a zone of inhibition on agar plates of staphylococci bacteria. He went on to show that the culture filtrate, named penicillin, showed activity against both Gram positive and negative bacteria and demonstrated some *in vivo* activity as a topical antibiotic. However, the true utility of penicillin as a cure for systemic infections was not discovered until 1940 by Florey and others at the School of Pathology at Oxford. With the war effort overwhelming most of the UK pharmaceutical industry, penicillin was not considered a sufficient priority to undertake the huge task of optimising large-scale production and carrying out clinical testing of penicillin. Seeing its massive potential, however, Florey convinced several American pharmaceutical companies to work towards its production and after two years of intensive efforts sufficient penicillin could be produced to satisfy the Allies' military efforts during the invasion of occupied France in 1944.

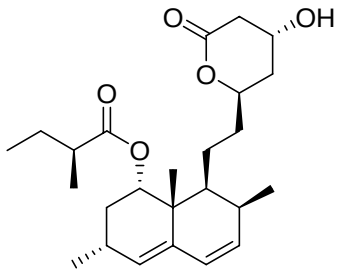
As well as being a hugely important drug in itself, the success of penicillin led to the large-scale scientific examination of fungi and other organisms as a source of potential drugs; an effort which led to many further breakthroughs in medicine. This was complementary to the rapid improvement in our understanding of disease and its causes. The development of increasingly specialised assay systems for a range of diseases allowed natural products, natural product derivatives and purely synthetic compounds to be screened for specific purposes, leading to more advanced drugs. Some examples are given below.

During investigations of fungal metabolites for antimicrobial activity, scientists at Sandoz in Basel discovered the peptide cyclosporin A, **1.5**.[6,9] This compound displayed only moderate anti-fungal activity; however, its true value was found when its potent immunosuppressant activity was discovered. It rapidly revolutionised the field of organ transplant by radically reducing the organ rejection which was the main cause of mortality in organ replacement patients.



1.5

The most profitable drug class to come from natural product sources is undoubtedly the serum cholesterol-lowering statin drugs.[6,9,11] High levels of cholesterol is a key risk factor in coronary heart disease and the discovery of a natural product, lovastatin, **1.6**, which could inhibit the *in vivo* biosynthesis of cholesterol provided an excellent prophylaxis. Lovastatin and many other natural and synthetic analogues now form a highly profitable class of drugs which has had a large impact on the treatment of heart disease.



1.6

This brief survey of the history of natural products as medicines is in no way intended to be exhaustive, with only a selection of examples of the way natural products have synergistically developed alongside medical understanding. The modern pharmacopeia has extended the life expectancy of many populations massively. However, it is worth noting that the World Health Organisation estimates that around 80% of the world's inhabitants still rely largely on traditional medicines for their primary healthcare.[7]

1.3 The influence of natural products on drug discovery

It should be clear from the above that the field of natural products research has played a critical role in the development of modern medicine. Despite the rise of synthesis, and in particular combinatorial chemistry, in drug design, natural products continue to play an important role to this day. It is impossible to quantify this impact, however some statistical analyses on the topic have appeared in the last ten years, covering the period from 1981 to today.[7,12,13] These analyses show that of all the new small molecule chemical entities approved as drugs in this time period, 39% were either natural products, directly derived from natural products or synthetic compounds whose design was based on a natural product pharmacophore.[13] In the areas of anti-infectives and anti-cancer drug discovery, natural product research has been particularly productive. Amongst anti-cancer drugs (approved between the 1940's and today), 57%, and of anti-infectives 63% (approved between 1983 and 1994) were natural products, natural product-derived or synthetic analogues of natural product pharmacophores.

The reasons for the success of natural products as drugs and in inspiring drug design has been the subject of much debate, particularly since the rise of their main competitor in the field - combinatorial chemistry.[14,15] There are a number of features of natural products which make them good potential candidates as phamaceuticals.[16] First, the very reason for the production of secondary metabolites by organisms is to interact in some way with other biological entities in order to confer an advantage to the producer. Thus, natural products can be thought of as 'drugs' which have been fine-tuned over thousands or even millions of years by nature to interfere with biological processes. This leads to massively diverse groups of highly ornate, stereodefined, rigid entities which only the most ambitious of combinatorial chemists could ever hope to mimic. Secondly, natural products are generally produced to interact with other organisms and as such they must have a proven degree of environmental stability, bioavailability and overall 'pharmacoviability' in nature. Whilst the biological settings for a pharmaceutical may be very different from those in their original setting, this pharmacoviability may be a case of 'half the battle won'.

However, there are a number of clear disadvantages associated with natural products as a source of drug leads. These relate mainly to identification of the active natural product, and issues of supply. The use of large libraries of extracts from various natural sources against high-throughput assay systems often brings to light potential drug leads. However, once an extract has been selected, identification of the active component in what may be a very complex mixture of compounds can be difficult. The complexity of natural products can make elucidation of the structure of the active component an arduous task. Once the active component has been identified, reasonably significant quantities of this natural product are required for initial biological evaluation, and much more will be required for *in vivo* testing and clinical supply if the compound is successful. Most natural products are produced on a very small scale by their host organisms and isolation of the compound of interest directly from the natural source is often (although not always) uneconomical on large scales. A number of approaches have been used to circumvent these supply issues: synthesis and, more recently, the use of natural product expression in heterologous hosts is being explored (discussed further later), however it is worth noting that these approaches are not without their own problems.

In light of these disadvantages, combinatorial chemistry seemed an excellent alternative source of drug leads. The huge libraries of purely synthetic compounds produced by combinatorial chemistry ensured that both the structure of any potential drug 'hits' was already known, and that a supply was readily available by already validated synthetic routes. However, a number of problems have become evident when looking for 'hits' amongst these libraries. First, these libraries were prone to false positives if any trace of reagents, side-products or other impurities were present in the samples. Secondly, the number of new drug leads found from these libraries was significantly lower than had been anticipated. Attempts to explain this phenomenon invoke the concept of the quality or pedigree of the compound library. As discussed above, natural products libraries are typified by structurally highly diverse and complex groups of compounds, whereas combinatorial libraries tend to occupy a smaller area of what has been described as chemical space.[14] Chemical space is an abstract concept which attempts to combine a variety of properties of a compound such as molecular weight, number of stereogenic

centres, rings, heteroatoms and solubility. Since combinatorial libraries are generated by variance of substrates in a matrix of common reactions, the diversity in chemical space of the resultant library is limited. It has been noted that drugs occupy a very large chemical space, as do natural products, which is why natural product libraries have a higher 'pedigree' when searching for potential drugs.

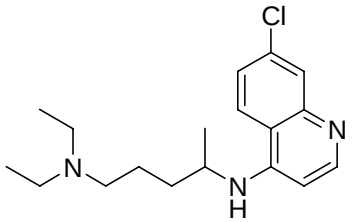
The intelligent marriage of these two approaches is likely to be the most useful approach for future drug discovery programs. Few would claim that natural products no longer have a role to play in drug discovery. The massive improvement in analytical techniques continues to simplify the elucidation of natural product drug leads. In those cases where supply issues are critical to the success of the emerging pharmaceutical, total synthesis or the synthesis of more accessible natural product analogues will continue to facilitate the viability of natural products as lead compounds in drug discovery.

1.4 The influence of natural products on organic synthesis

In addition to the important role the field of natural products has had on drug design, it has also influenced other spheres of science, in particular the field of organic synthesis. These two fields have developed synergistically, with developments in one field pushing the boundaries of the other.[16]

The aim of organic synthesis is to generate 'organic' compounds, or in other words, the compounds derived from living organisms, from simple building blocks. The synthesis of urea, an organic compound, from inorganic ammonia and cyanic acid by Friedrich Wöhler in 1828 is often heralded as the demise of vitalism and the beginning of the field of organic synthesis. In fact this is a popular myth and it has been argued that it is historically more appropriate to attribute the demise of vitalism to Kolbe's synthesis of acetic acid.[17,18] The early years involved the synthesis of very simple organic molecules, but the 20th century saw the interest in, and the sophistication of synthetic chemistry increase exponentially. This occurred simultaneously with the improved analytical

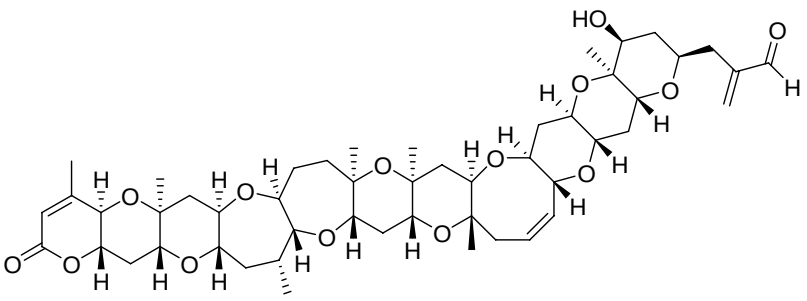
techniques which allowed the structures of increasingly complex natural products to be elucidated. The synthetic targets chosen were often driven by interest in the biological activity of a natural product whose supply was questionable. An example is the total synthesis of a quinine precursor by Woodward and Doering in 1944.[19] The supply issue of this strategic drug led to intense interest in a possible synthetic source. When the successful synthesis was announced there was much fanfare amongst the public, although realistically the synthesis could not have provided a genuine alternative source, and the careful management of natural resources and discovery of synthetic analogues such as chloroquine, **1.7**, made the synthesis of quinine, **1.1**, a more academic pursuit.



1.7

Although the synthesis of quinine did not solve its supply issues, it did show that the synthesis of complex, biologically interesting natural products could be achieved. Early synthetic conquests were largely achieved by clever exploitation of known reaction chemistry.[16] The burgeoning field of natural products was, however, yielding ever more complex targets with interesting biological activities, on which the fresh faces of synthetic chemistry could test their mettle.[20] In order to synthesise many of these new compounds, the methodology available to synthetic chemistry had to increase radically. The explosive growth in synthetic methodology in the last half century is attributable to the inventiveness of synthetic chemists, determined to reach their chosen natural product targets. New methods involved both the classic elements with which organic chemists were well acquainted (eg C, H, N, O, the halogens and alkali earth metals), as well as exploitation of other elements (eg B, S, Se, Si, Sn, P) and in particular the transition metals, which opened up new synthetic opportunities.[16] With the development of the new methodology, yet more ambitious natural products could be targeted, leading to further methodology development. In this way, the field of organic synthesis became an increasingly sophisticated art, constantly being pushed by natural product discovery. The complexity of the natural products which can now be successfully targeted (eg brevetoxin

B, **1.8**;[21] palytoxin, **1.9**)[22,23,24] would have astonished the early pioneers in this field (and frankly, astonish this author).



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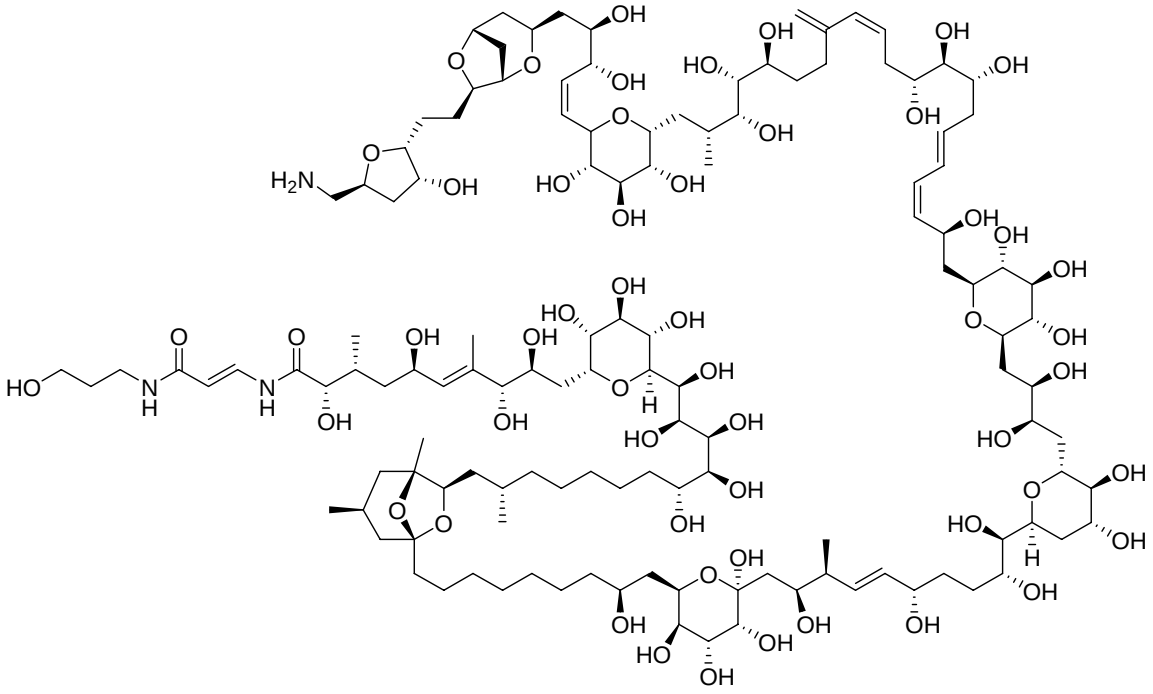
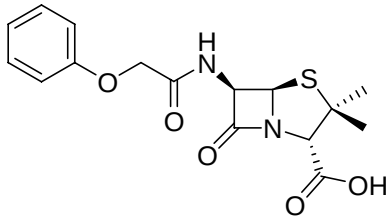


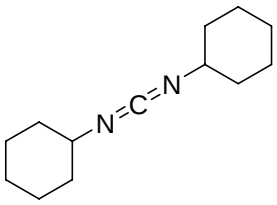
1.9

An early example of the advances in methodology which resulted directly from the synthesis of a natural product is the development of mild dehydrating agents by Sheehan, used in the synthesis of penicillin V (**1.10**).[25,26] Although fermentation methods were able to generate significant amounts of penicillin for the war-effort (*vide supra*), the synthesis of penicillin was also actively pursued. The synthesis of the penicillins had been hampered by an inability to form the highly labile β-lactam moiety which was so

critical to the activity of these natural products. All the dehydrating agents for amide bond formation of the day involved harshly acidic conditions (eg thionyl chloride and phosphorous trichloride), and Sheehan appreciated that a more mild system would be required for the sensitive penicillin skeleton. His development of dicyclohexylcarbodiimide (**1.11**) as a mild and efficient dehydrating agent allowed the synthesis of this challenging target to be completed. In addition, the development of a mild dehydrating agent for amide bond formation had a huge effect on the field of peptide synthesis and aided important new developments such as solid-phase peptide synthesis.



1.10



1.11

The increased sophistication and scope of the new methodologies gleaned from natural product synthesis also impacted on other areas of chemistry. From inorganic chemistry to biochemistry, materials science to nanotechnology; all have benefited from the large synthetic repertoire developed. Ironically, it is on the back of the wealth of robust new methodology, increasingly well validated and understood, that combinatorial chemistry now rides. The huge libraries of combinatorial chemical entities could not have been made without the hard work of countless synthetic chemists racing to be the first to publish the total synthesis of the latest natural product.

Evidently, other branches of chemistry have played their roles in the development of synthetic chemistry, but only the field of natural products can claim to have challenged and forced the imagination of synthetic chemists; cajoled them even, into pushing the boundaries of their science ever further.

1.5 The influence of organic synthesis on natural products

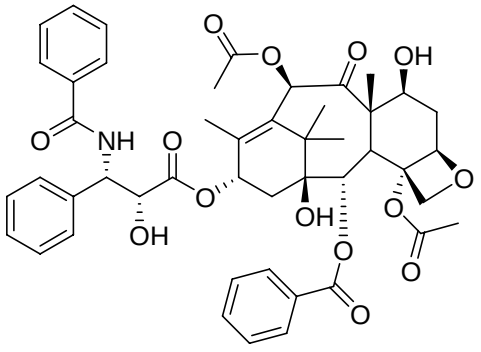
As mentioned earlier, the development of the field of natural products and organic synthesis was synergistic. In **Section 1.4** the importance of natural products to the development of organic synthesis was discussed. Here, the importance of organic synthesis to natural product research will be examined.

There are three main ways in which synthesis has aided natural product research; firstly in the validation of proposed structures for new metabolites, secondly by allowing improved supply for metabolites produced in insufficient quantities, and thirdly by generating analogues of the natural product pharmacophore which are, in some way, better than the original natural product.

During the early years of natural product research, the structural elucidation of metabolites largely relied on extensive degradation and/or derivatisation studies, which allowed the researcher to piece together the structure. This pain-staking process required a great deal of deductive skill and patience on the part of the researcher. The unambiguous proposal of a single structure was often difficult or impossible. The final validation therefore usually required the total synthesis of the proposed structure and comparison of physical data. With the development of a number of analytical techniques, most notably X-ray crystallography, NMR spectroscopy and mass spectrometry over the last half century, the art of structure elucidation has been transformed. It is now possible, and even routine, to elucidate the structure of very complex molecules, including their stereochemistry, present in milligram or even sub-milligram quantities. These massive advances in technology would suggest that virtually any metabolite will succumb to elucidation without hitch, removing the need for any validation of the proposed structure. Whilst it is true that the vast majority of newly elucidated natural products are accurately proposed by the careful use of these techniques, a not insignificant number of structures are subsequently shown to have been misassigned.[27] These misassignments are regularly only recognised on completion of a total synthesis.[28,29] The misassignments are generally not the result of sloppy work on the part of the researcher, but the result of an unexpected

ambiguity in the data.[27] This demonstrates the continued value total synthesis has in validating natural product structures.

One of the chief complaints about natural products in drug discovery is the issue of supply. The sustainable production of sufficient drug to supply demand in an economical fashion is a critical factor in the viability of any pharmaceutical candidate. Many drugs derived from microorganisms can be successfully generated by fermentation eg daptomycin.[30] However, total synthesis also plays an important role in alleviating the problem of supply. Galantamine (Alzheimer's) and mycophenolate sodium (immunosuppression), two natural product drugs which have recently reached the market, are both produced by total synthesis.[31] Semi-synthesis is another approach to alleviating problems of supply, with paclitaxel or taxol (**1.12**) being a good example. This clinically important anti-cancer drug is a natural product originally isolated from the bark of the Pacific yew tree (*Taxus brevifolia*) and its potential was recognised during research in the 1960s and 70s.[32] However, only 1 g of the metabolite could be extracted from 24 kg of dried bark. Since a 125 year old tree was only capable of producing around 2 kg of bark and this species was relatively rare, the issue of supply was particularly acute in the case of this natural product and slowed its development as a drug. The total synthesis of taxol was completed by Holton and Nicolaou independently.[33,34,35] However, it was taxol's semi-synthesis from a natural congener, 10-deacetyl baccatin III (**1.13**), more sustainably obtained from the needles of the *Taxus baccata*, which allowed for its large-scale manufacture and commercial supply.[36]



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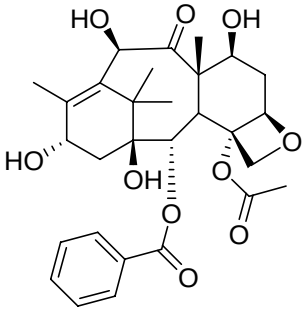
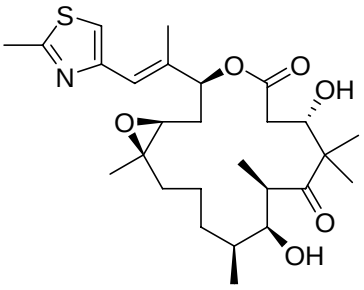


1.13

Finally, synthetic studies can be used to improve the natural product pharmacophore in some way. This may be by directly improving an aspect of a natural product's biological activity, for example by reducing toxicity, increasing potency or selectivity or improving bioavailability. Alternatively, synthesis may lead to an improvement in the economy of production. A combination of these factors was achieved during the synthetic studies of epothilone B (**1.14**) by Danishefsky and co-workers, leading to several very promising lead compounds, which are currently at various stages of clinical testing.[16,37]



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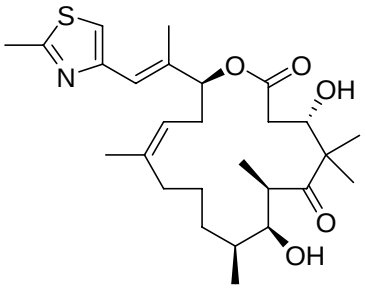

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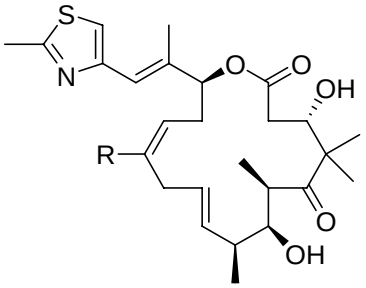

1.16 R=CH_3
1.17 R=CF_3

The highly cytotoxic epothilone B displays activity against multi-drug resistance (MDR) cancer cell lines, which sparked interest in its synthesis. Having successfully synthesised the natural product, the group of Danishefsky then carried out diverted total synthesis (DTS) on the pharmacophore. DTS can be defined as a process of optimisation of drug leads from natural products by using intermediates generated during the total synthesis of the natural product as a platform to generate a range of related analogues. By examining these related analogues, any improvements in the pharmacophore will be discovered and a structure-activity relationship developed. An understanding of medicinal chemistry

may allow the focussed design of this small library such that specific characteristics are added to, or removed from the pharmacophore. In DTS studies on epothilone B, the removal of the epoxide of the natural product yielded an analogue (**1.15**) which is better tolerated *in vivo* due to the removal of the non-selective biological activity of this motif (currently in phase II testing, May 2006). Whilst this product retained activity against MDR cell lines, its potency was less than that of **1.14**. The authors then carried out further DTS studies and found that 9,10-dehydro-dEpoB (**1.16**) and its trifluoromethyl analogue (**1.17**) retained selectivity, but showed potency analogous to that of the natural product. Analogue **1.17** shows particular promise as an anti-cancer drug, with low toxicity and excellent pharmacokinetics, as well as high activity, and entered preclinical trials (May 2006).

The synergistic development of synthetic chemistry and natural products research has therefore been to the great benefit of both fields, as well as many other fields of science.

1.6 Future trends

Investment in natural product programs by pharmaceutical companies was seriously reduced during the last twenty years as emerging technologies, in particular combinatorial chemistry, became popular. Scientists have expressed surprise that such a time-honoured and successful approach had been abandoned so readily for far less validated drug discovery platforms.[16] In more recent years, however, natural products have experienced a resurgence of interest from the pharmaceutical industry, and there is an increasing realisation that a combination of natural product libraries, more focussed combinatorial chemistry, and rational design will provide the best opportunities for drug discovery.[15,38]

Synthesis is likely to continue to feature heavily in drug development programs. The increased interest in and success of "green" chemistry is likely to improve the sustainability of using synthesis for commercial pharmaceutical supply.[39]

The use of computational tools, such as virtual screening of *in silico* compound libraries for potential drug leads and for rational lead optimisation will, no doubt, continue to be important in drug design.[40] Computing power continues to increase rapidly, and equally, scientist's ability to design appropriate models and critically analyse results improves with experience, which is leading to more reliable predictive capabilities.

As well as improvements in these traditional technologies, new technologies are also emerging which may aid pharmaceutical design and viability.[41] In particular, the increased understanding of the enzymes involved in secondary metabolite production, and the genes which code for them, is opening up the opportunity of 'combinatorial biosynthesis'.[42,43] The polyketide and non-ribosomal peptides are the classes of natural products that are most amenable to this technology, and are two of the most productive biosynthetic pathways of viable pharmaceutical lead compounds. Both classes are biosynthesised by either one or a small collection of large, modular synthetases (additional tailoring enzymes may also be involved). These act as factory lines, with each well-defined module carrying out a catalytic reaction and passing the natural product precursor onto the next module for further manipulation, until the completed metabolite is released by the enzyme. It has been found that these modular enzymes are highly promiscuous. Not only can they be fed different precursors, leading to subtle changes in eventual metabolite structure, but the constituent modules can even be manipulated, deleted or exchanged, leading to radical variations in the structure of the final metabolite. Not only can this approach lead to libraries of novel 'natural' products,[44,45] but those which prove to be good drug leads can be produced on large scales since the genes coding for them can be introduced into heterologous hosts which are amenable to large-scale fermentation.

In an alternative but related approach, 'combinatorial biology' offers the possibility of screening natural products produced by unculturable microorganisms, radically expanding the portion of biodiversity that can be exploited for pharmaceuticals.[41] This is carried out by isolating and cloning the DNA from problematic microorganisms into

heterologous hosts. The natural products produced by these organisms can then be expressed and examined for biological activity.

The future of medicinal chemistry will therefore continue to be a very interdisciplinary venture. The combination of talented chemists, biochemists, microbiologists, not to mention physicians will ensure that our understanding of the causes of disease will deepen and our ability to treat or prevent disease will improve. The issue of sustainability will also be of increasingly critical importance, as competition for resources, particularly petrochemicals, becomes more intense. The use of fermentation processes using renewable biomass may therefore become increasingly economical, and indeed ethical.

1.7 Aims of this thesis

The primary aim of the work presented in this thesis was to utilise synthesis as a tool in the study of natural products isolated by the natural products research group at the University of Canterbury. In particular, the natural products chosen would be of biological interest, as determined in this group's in-house biological assays.

The synthetic studies undertaken covered two different stages in the development of natural products as potential lead compounds in drug discovery. The first project utilised synthesis as a tool in the elucidation of the structure of natural products, namely the pteratides.[46] The second project endeavoured to establish a total synthesis of a natural product whose structure had already been assigned, *spiro*-mamakone A.[47] The latter would also serve as a platform for diverted total synthesis, allowing the generation of a library of natural product analogues and therefore, enabling the beginnings of a structure-activity relationship to be established.

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Chapter 2

The Pteratides

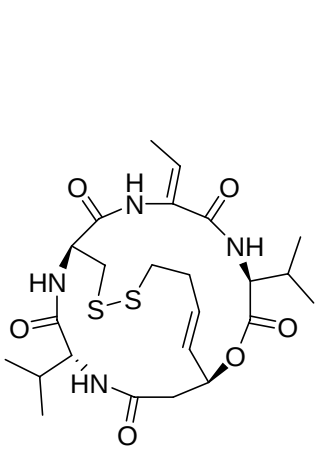
2.1 Introduction

2.1.1 Depsipeptides

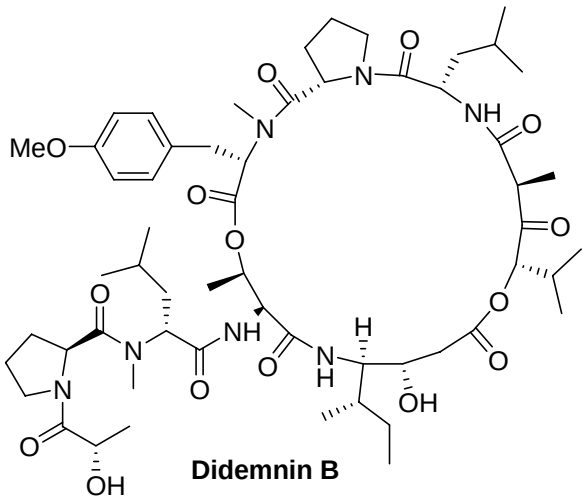
Polypeptides are a central motif in biological systems, and constitute more than 50% of the dry weight of cells. The overwhelming proportion of these are proteins, which play a crucial role in primary metabolism by catalysing the chemistry required for life. Certain proteins can also provide essential structural components of cells, such as actin, whilst others, such as insulin and angiotensin II, regulate cellular processes. They are unbranched polymers of the 20 common amino acids, linked by amide bonds and are formed by ribosomal peptide synthesis.

In addition to these 'ribosomal peptides', peptides can also be produced by 'non-ribosomal peptide synthesis', which involves dedicated biosynthetic pathways producing very diverse peptides for specific roles. These fall into the category of secondary metabolites. Amongst these, it is common to find amino acids incorporated which are either common amino acids which have been chemically modified, or even amino acids entirely distinct from the 20 common amino acids. Biosynthetically distinct groups, such as polyketides, can also be incorporated into the peptide chain. Backbone linkages other than amide bonds can be formed, in particular ester linkages to give a class of peptide called depsipeptides. Cyclic peptides are also common,[1] with cyclisation generally occurring via an amide linkage between termini of the peptide, or between one terminus and an appropriate amino acid side-chain functionality. In those instances where cyclisation occurs with a side-chain hydroxyl or phenol group, forming an ester linkage, the name cyclodepsipeptide is used.

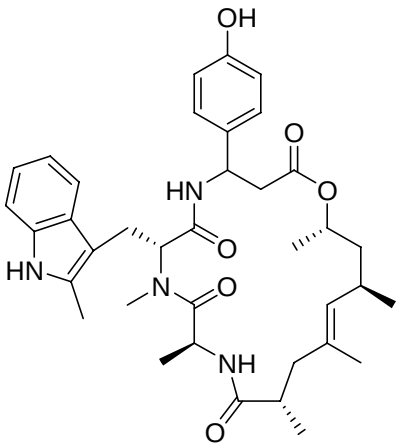
Many cyclodepsipeptides show promising biological activities. Examples are didemnin B and FR901228 which have entered clinical trials as anti-cancer agents and the jaspamides which show a variety of biological activities including cytotoxicity, antimicrobial and antifungal properties.[2]



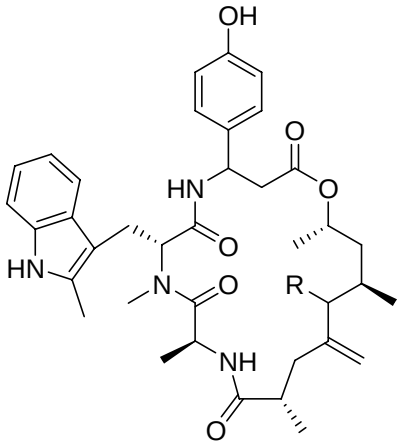
FR901228



Didemnin B



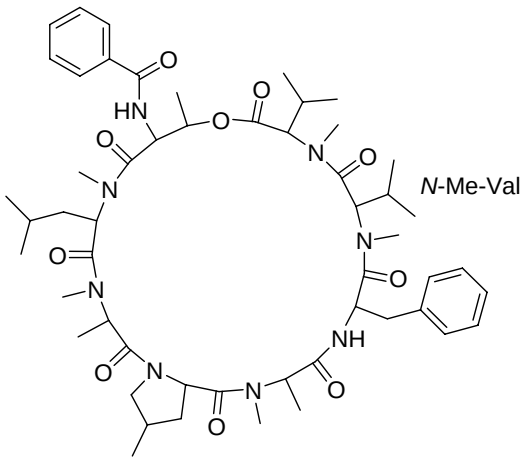
Jaspamide A



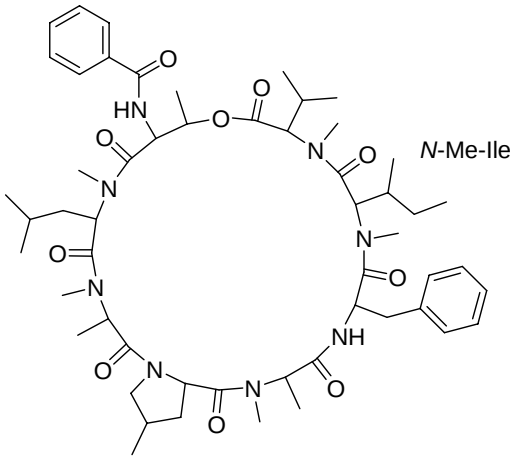
Jaspamide B R =O
Jaspamide C R -OH

2.1.2 Pteratides

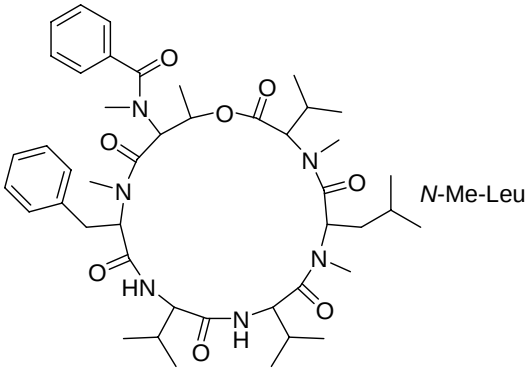
The pteratides are a new class of highly cytotoxic cyclodepsipeptides recently discovered by Miss C-H Chen of the Marine Group at the University of Canterbury.[3] The family is comprised of three depsipeptides; two octadepsipeptides (pteratides I and II) and one hexadepsipeptide (pteratide III). They were isolated from the fruiting bodies of a coral-shaped Malaysian basidiomycete, identified as a *Pterula* species (**Figure 2.1**).



Pteratide I



Pteratide II



Pteratide III

Figure 2.1 – Malaysian basidiomycete, *Pterula* species; the source of the pteratide series of natural products

Pteratides I and II display potent cytotoxicity against the P388 murine leukaemia cell line (IC_{50} values of 41 and 40 nM, respectively), whilst pteratide III shows more modest, but still notable, activity (2.9 μM).

The structures of the pteratides I to III were elucidated by Miss Chen using extensive NMR and mass spectral analysis. ^{1}H and 2D NMR data were used to establish the constituent amino acids and connectivity. Methanolysis and ESI MS/MS of the resultant methyl esters were carried out on pteratides I and II, and the fragmentation pattern observed confirmed the proposed connectivity.

The pteratides are structurally very interesting; incorporating many 'unnatural' or 'non-coded' amino acids (those not amongst the 20 coded amino acids recognised by tRNA). Pteratides I and II each include five *N*-methylated amino acids, and pteratide III includes four.

N-Methylation is a common chemical modification found in peptide secondary metabolites and is introduced by tailoring enzymes on the growing peptide chain during biosynthesis.[4,5] As secondary amines, *N*-methylated amino acids introduce different structural features to the backbone of a peptide, in a manner similar to that of proline, the only secondary amine of the common amino acids. Secondary amino acids confer structural rigidity to the peptide backbone as well as promoting the less favoured *cis*-conformation about the amide bond (discussed further in **Section 2.2.2**). *N*-methylation of the backbone may alter intramolecular hydrogen bonding sites, which also alters the conformation of the peptide. As such, *N*-methylation is an important biosynthetic tool in establishing the three-dimensional shape of peptidic natural products.

N-Methylation of peptides also alters other physical properties important to biological activity. For example increased membrane permeability may be observed, due to the reduction in hydrophobicity,[6] and increased proteolytic stability, due to the blocking of enzyme cleavage sites.[7]

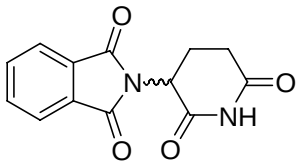
Another interesting structural feature of the pteratides is the incorporation of a benzoyl *N*-terminus capping group. This motif is relatively common, with examples including *N*-benzoyl-*S*-phenylalaninol and related natural products such as anabellamide and hypothallin.[8,9,10]

Finally, pteratides I and II incorporate the very unusual amino acid 4-methylproline. This amino acid was first isolated in young apples,[11,12,13] and has also been found in bacteria,[14,15] cyanobacteria,[16,17,18] the sponge *Theonella* sp.[19] and fungal sources.[20,21,22] This unusual feature is possibly the most interesting aspect of this series of natural products and elucidation of the importance of the methyl group to the biological activity observed in the natural products would be of critical value in a structure-activity relationship study.

2.1.3 Stereochemistry in biology

Chirality is important in biological systems as most of the building blocks of life contain stereogenic centres, and the correct spatial arrangement is important in interactions between biochemical entities. For example, recognition of substrates by enzymes and receptors is critically dependant on the stereochemistry of the substrate.

Many drugs are known in which one enantiomer is the active form, and the other is either inactive, or displays an entirely different activity. The classic example of such a drug is thalidomide, which was marketed for the relief of morning sickness, amongst other indications, to pregnant women in late 1950's.



thalidomide

The drug was given as a racemic mixture of its two enantiomers, but due to insufficient testing, the highly teratogenic properties of one of the enantiomers (levorotatory) was not

realised until after it had caused deformities in an estimated 10,000 children. It was subsequently discovered that racemisation occurs *in vivo*, such that even prescribing the enantiomerically pure, non-teratogenic form may not have prevented the tragedy.[23]

2.1.4 Stereochemical elucidation

With the nature of the amino acids present in the pteratides, and their connectivity already established, the remaining aspect of their structure to be elucidated was stereochemistry. Amino acids (with the exception of glycine) contain at least one chiral centre and all of the 20 common amino acids (except glycine) are found naturally in the L form in ribosomal polypeptides. However, as noted above, peptides of non-ribosomal biosynthetic origin regularly include non-natural amino acids, including those of the D series. It was therefore important to establish the stereochemistry of each of the amino acids to conclude the elucidation of the pteratide structures.

There are a number of standard procedures for the elucidation of the stereochemistry of amino acids, which involve the use of chromatography to separate individual isomers. HPLC has proved to be a powerful tool for the separation of small quantities of compounds and is the method of choice in this case. In order to study the stereochemistry of each amino acid, the peptides must first be broken down into their constituent amino acids, generally by hydrolysis of the amide (and ester, where present) bonds. The constituent amino acids can then be compared to reference materials of each possible stereoisomer of each amino acid, to establish which is present in the natural product.

Enantiomeric pairs cannot be separated on standard reverse phase columns since their physical properties are identical and therefore they will have identical affinities for the stationary phase. Both direct and indirect methods have therefore been developed to allow the separation of amino acid enantiomeric pairs. Direct methods involve the use of a chiral selector. This can either be directly attached to the stationary phase, creating a chiral stationary phase such as the Phenomenex Nucleosil® Chiral 1 column, or the chiral

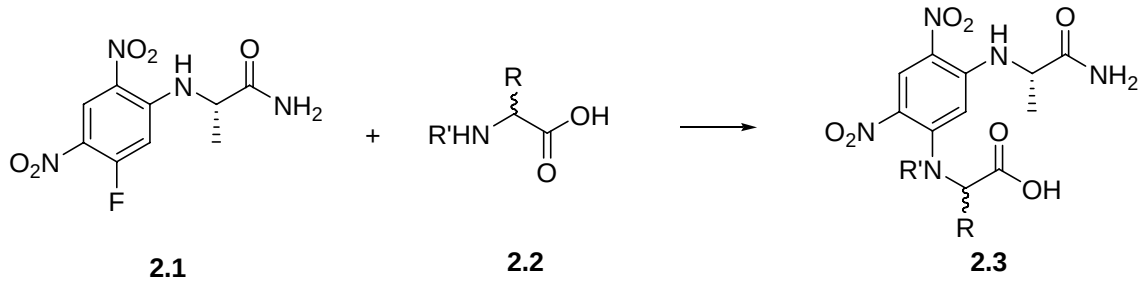
selector can be an additive in the mobile phase, eg L-histidine methyl ester/copper complex for separating dansyl amino acid derivatives.[24] Indirect methods involve a pre-column derivatisation step with a chiral reagent. The diastereoisomeric products formed can then be separated on standard reverse phase columns.

There are a number of advantages and disadvantages to the direct and indirect methods, some of which are summarised in **Table 2.1**.

Table 2.1 – Comparison of direct and indirect methods of enantiomeric purity analysis

	Direct enantiomeric purity analysis	Indirect enantiomeric purity analysis
Advantages	1. Simple sample preparation 2. Enantiomers have same UV adsorption so no detector bias 3. Racemisation is less likely during sample analysis 4. Purity of chiral selector not as critical	1. Resolution of enantiomers is often superior 2. Elution order can be easier to predict 3. Can be used to enhance sensitivity 4. Achiral columns cheaper and more rugged
Disadvantages	1. Resolution may be poor 2. Lengthy method development time may be required 3. Expensive columns or mobile phase additives required 4. Elution order often more difficult to predict	1. Enantiomeric purity of chiral selector important trying to detect a trace enantiomer 2. Diastereoisomers may not have same UV adsorption leading to detector bias 3. Racemisation during derivatisation may occur 4. Derivatisation steps may be time-consuming 5. Excess derivatisation reagent may interfere in chromatogram

A very popular pre-column derivatisation procedure for indirect enantiomeric purity analysis is the reaction of the amino acids with 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide, also known as Marfey's reagent, **2.1**.[25,26] The amino acid in question, **2.2**, either of unknown stereochemistry or a reference material, undergoes a nucleophilic aromatic substitution reaction with this chiral reagent, **2.1**, to form diastereomeric Marfey's derivatives, **2.3** (shown in **Scheme 2.1**).



2.1 **2.2** **2.3**

Scheme 2.1 – Derivatisation with Marfey's reagent

The Marfey's derivatives of the amino acids can then be separated, often with very high selectivity factors, on C_8 or C_{18} reverse phase columns. It has been noted that amino acids of the L series elute with an earlier retention time than the corresponding D series, however some exceptions to this general rule have been encountered. The derivatives have a distinctive UV chromophore (**Figure 2.2**), making identification simple.

The popularity of this particular indirect method lies in both its simplicity and its effectiveness. The derivatisation step is easy to carry out, and the yield of the reaction is usually in excess of 99% (around 2 equivalents of Marfey's reagent are commonly employed). No racemisation is observed during the derivatisation step and the hydrolysed reagent side-product does not generally coincide with the amino acid derivatives. The introduction of a strong UV chromophore at 338 nm allows for very low detection limits. This was the method chosen to study the stereochemical composition of the pteratides.

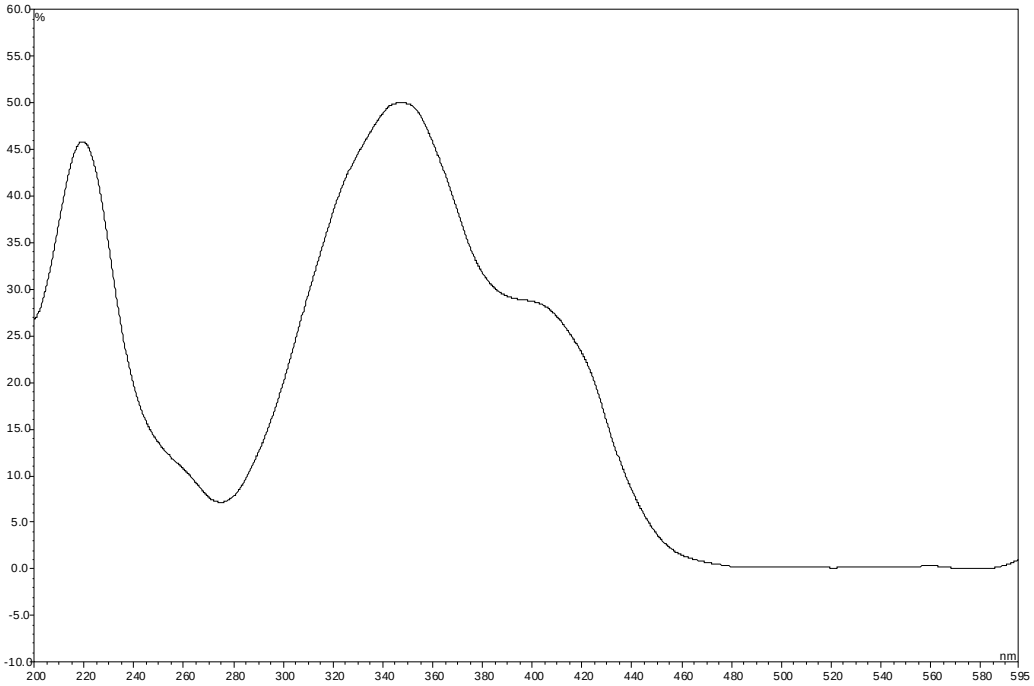


Figure 2.2 – UV chromophore of Marfey's derivatives

2.1.5 Reference materials

In order to establish the stereochemistry of the amino acids of the pteratides, reference materials of all possible stereoisomers of the amino acids in question must be available. All stereoisomers of the 20 common amino acids are available commercially, and many of the less common amino acids are also available. However, several of the amino acids found in the pteratides were either unavailable (in the case of 4-methylproline) or expensive.

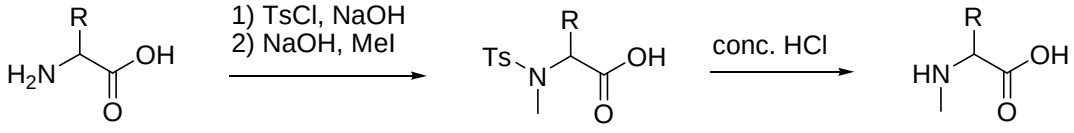
Most of the amino acids required were already available in house. However, *N*-methyl-L-valine, the four stereoisomers of *N*-methyl-threonine and the four stereoisomers of 4-methylproline were not. Initial work towards the elucidation of the stereochemistry of the pteratides therefore involved the synthesis of these amino acids. These syntheses were carried out by directly following literature precedent, or modifications thereof, and are discussed in the following sections.

2.2 Synthesis of *N*-methyl amino acids

2.2.1 Introduction

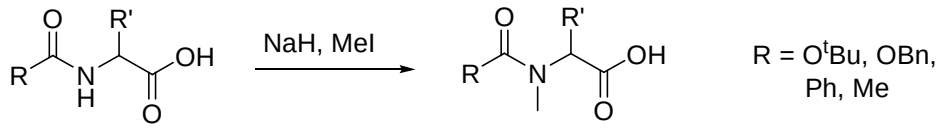
N-Methylated amino acids have been highly sought-after synthetic targets whose preparation has been explored since Fischer's first pioneering work.[27] Their regular appearance in natural products and the interesting properties their presence imparts on the biological activity of peptides has ensured active research continues on their synthesis. Increasingly mild techniques have been sought as *N*-methylated amino acids which are protected or present in a peptide chain are more prone to racemisation under both basic and acidic conditions than their non-methylated counterparts. This is postulated to be because in the absence of ionizable groups other than the α-centre, this centre is left exposed to racemisation.[28,29] A number of varied approaches have been studied,[30] a brief overview of which will be given here.

Emil Fischer was the first chemist to prepare *N*-methylated amino acids using the direct methylation of tosyl derivatives of amino acids, with methyl iodide and base (**Scheme 2.2**).[27] Sulfonamide protection of amines greatly enhances the acidity of the sulfonamido nitrogen, due to the strongly electron withdrawing nature of this group, allowing deprotonation and subsequent attack on an alkyl electrophile. The strongly basic conditions involved in the methylation can cause significant racemisation, however, and the vigorous conditions required for *N*-tosyl deprotection also detract from this first success.[31] Milder conditions have been found, and of note is the methylation of *N*-nosyl amino acids with diazomethane under neutral conditions.[32,33] The *N*-nosyl (*p*-nitrobenzenesulfonamide) group is also a strongly electron withdrawing protecting group, but can be cleaved under milder conditions than the *N*-tosyl group.



Scheme 2.2 – Fischer's route to *N*-methylated amino acids

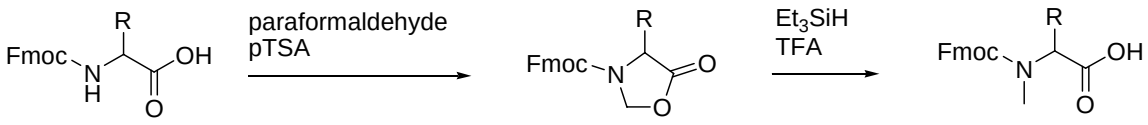
The *N*-methylation of *N*-acyl and *N*-carbamoyl amino acids has been successfully achieved, notably using sodium hydride and methyl iodide, as developed by Benoiton *et al*. (**Scheme 2.3**).[34] This method is a relatively mild procedure, although conditions must be carefully controlled to avoid racemisation. Variations of both the base[35] and methyl donor[36] have also been published.



Scheme 2.3 – Benoiton's route to *N*-methylated amino acids

Reductive amination is an attractive route to *N*-methyl amino acids due to the mild conditions involved. Whilst mono-alkylation of amino acids is possible using aldehydes larger than formaldehyde, [37] clean mono-methylation is not possible. The secondary amine produced is more nucleophilic than the parent primary amino acid, and competes for the aldehyde, leading to a mixture of starting material, mono- and di-methylated products. In the case of larger aldehyde homologues the additional steric bulk is sufficient to prevent the secondary amine from competing for the aldehyde. Multi-step routes have been published which achieve mono-methylation via reductive amination,[38] however, and these will be discussed later (**Section 2.2.3**).

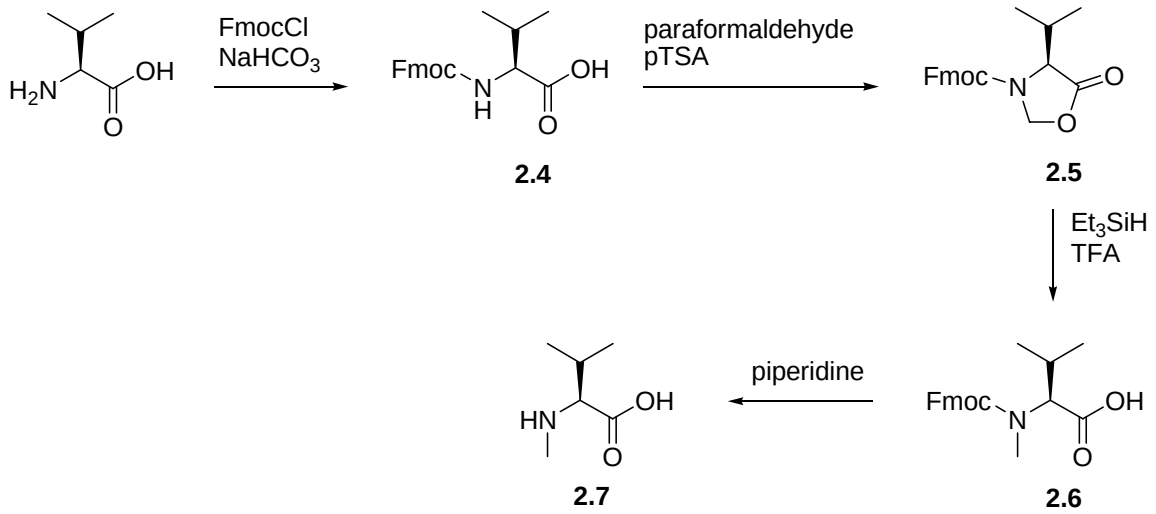
The final major route for *N*-methylation involves the intermediacy of oxazolidinones. These were first prepared from *N*-CBz amino acids by Ben-Ishai by an acid catalysed dehydration with paraformaldehyde, followed by intramolecular ring closing.[39] Freidinger applied this to Fmoc protected amino acids and established that reduction of the oxazolidinone ring furnished *N*-methyl-Fmoc protected amino acids (**Scheme 2.4**).[40] This route is very mild and no racemisation is observed. It was also found that these two steps (oxazolidinone formation/reduction) could be combined in one step.[41]



Scheme 2.4 – Freidinger's route to *N*-methylated amino acids

2.2.2 Synthesis of N-*methyl-*L*-valine*

In the synthesis of *N*-methyl-L-valine, it was decided to follow the method of Friedinger discussed above (**Section 2.2.1**) since it is a short, mild and high yielding synthesis.



Scheme 2.5 – Synthesis of *N*-methyl-L-valine

The synthesis (**Scheme 2.5**) begins with the Fmoc protection of L-valine under Schotten-Baumann conditions. This proceeded cleanly, to give **2.4** in a yield of 66%. The next step involves the formation of the oxazolidinone. The dehydration and cyclisation steps occur with paraformaldehyde and catalytic amounts of pTSA under standard Dean-Stark refluxing toluene conditions. A good yield of 94% of oxazolidinone **2.5** was obtained and the product was characterised by NMR and IR spectroscopy. The ^{1}H NMR chemical shift data compared well to the literature data, however a broadening of all peaks except those of the Fmoc group was observed. This broadening of the peaks can be explained by the slow interconversion of more than one ring conformation, and may be exacerbated by the presence of carbamate *cis-trans* isomerisation. In the amide bonds of standard amino acids (except proline), the *trans*- isomer is preferred as it minimises the steric interactions of adjacent amino acids. The energy barrier of conversion from *trans*- to *cis*- is also high, due to the significant double bond nature of the C-N bond, arising from the delocalisation of the nitrogen lone pair over the amide moiety. In the case of proline and other

secondary amino acids, the *trans*- preference is less pronounced as the second group on the amine introduces other steric interactions. The broadening of peaks was not, however, observed in the literature report. This may be due to a difference in the temperature at which the spectrum was carried out (23ºC, in this work; temperature not stated in the literature report. $CDCl_3$ used as solvent in both cases). Despite this ambiguity, the presence of the characteristic, strong oxazolidinone carbonyl IR absorption at 1801 cm^{-1} provided confirmation that the ring had been formed.

Reductive ring opening was carried out with triethylsilane, with chloroform and trifluoroacetic acid as the solvent. The *N*-methyl product, **2.6**, was obtained in 77% yield. NMR characterisation again showed minor variations on that reported in the literature. A doubling of peaks with a ratio of around two to one was observed in both the ^{13}C and ^{1}H NMR spectra. This can be readily explained by *cis-trans* isomerisation of the carbamate amide bond, as discussed above. It is surprising, however, that the same phenomenon was not noted in the literature report. High resolution mass spectral data confirmed the molecular formula of the product.

Finally, cleavage of the Fmoc group was carried out using piperidine to give the free *N*-methyl amino acid, **2.7**, in quantitative yield. NMR spectroscopic, high resolution mass spectral and optical rotation data were all in accordance with literature data. To confirm optical purity, Marfey's derivatives of the synthetic *N*-methyl-L-valine and commercial *N*-methyl-D/L-valine were carried out. Results of HPLC analysis of the derivatives (**Figure 2.3**) show that the synthetic *N*-methyl-L-valine was free from any trace of its enantiomer.

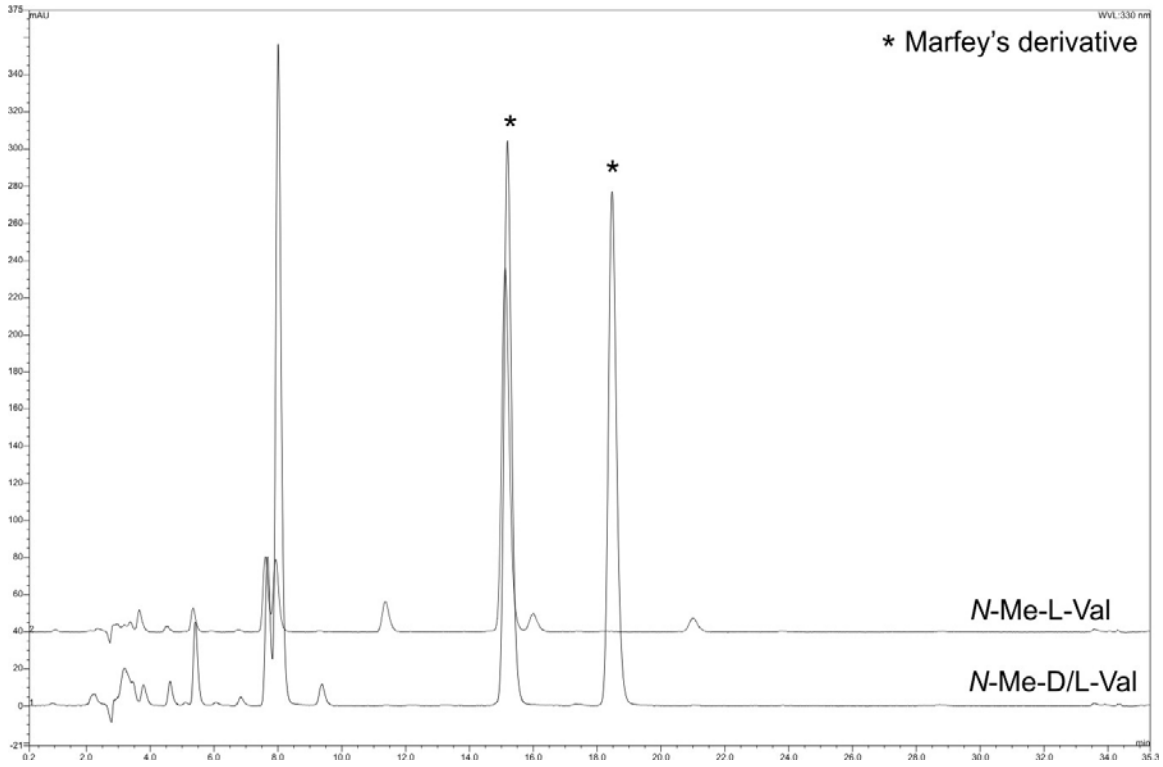
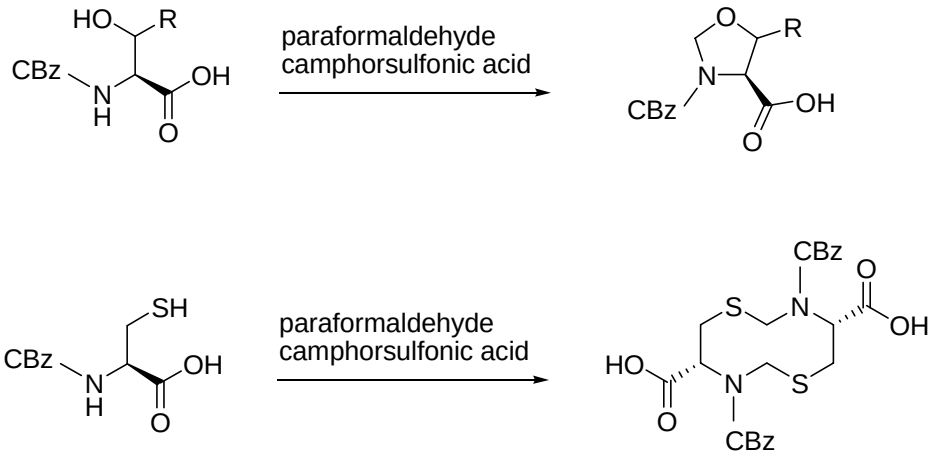


Figure 2.3 – HPLC analysis of the Marfey's derivatives of synthetic *N*-methyl-L-valine and commercial *N*-methyl-D/L-valine

2.2.3 Synthesis of N-*methyl-L- and D-threonine*

With *N*-methyl-L-valine in hand, attention was next given to the synthesis of all four diastereoisomers of *N*-methyl-threonine.

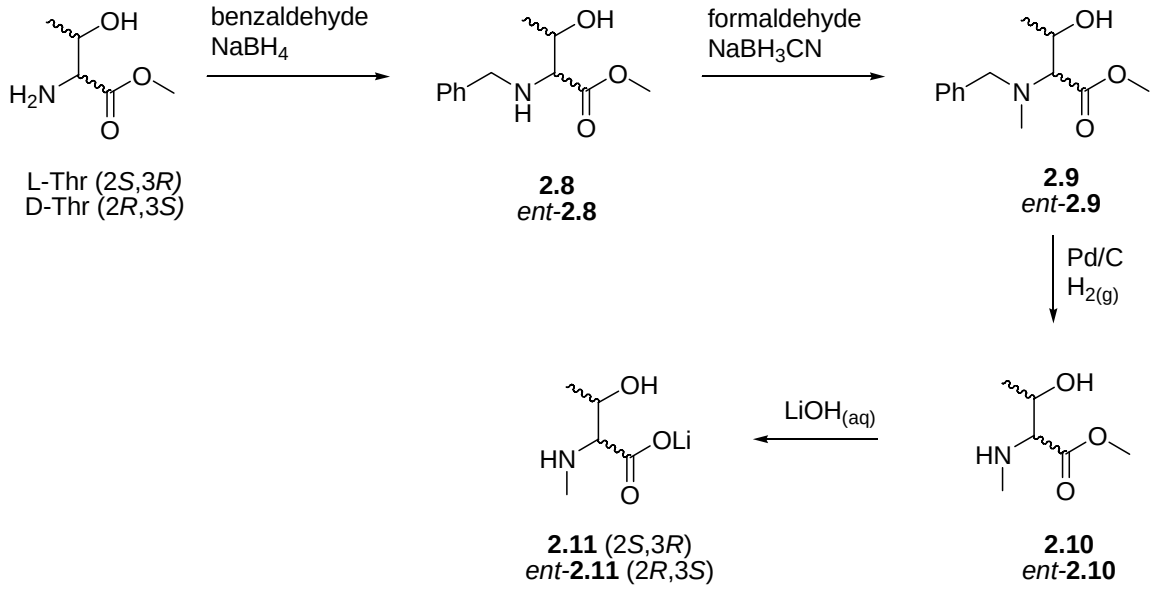
The procedure of Freidinger utilised to synthesise *N*-Me-L-valine (**Section 2.2.2**), via the intermediacy of an oxazolidinone has one crucial drawback. It works very well in the case of those amino acids with simple alkyl side-chains, however, certain side-chains are capable of intercepting the intermediate in oxazolidinone formation, creating alternative ring systems.[42] In the case of threonine and serine, the oxazolidine was the predominant product, and in the case of cysteine a dimeric product was observed (**Scheme 2.6**).



Scheme 2.6 – Major side-products of oxazolidinone formation in amino acids with reactive side-chains

Whilst this problem can be avoided with the use of side-chain protection, this adds two additional steps to the synthesis and makes it a less attractive route.[43] There are a number of other routes (**Section 2.2.1**), and a method for efficiently *N*-methylating threonine was reported by Boger *et al*.[38] This method involves the use of reductive amination to introduce the methyl group.

As discussed previously (**Section 2.2.1**), reductive amination of amino acids with formaldehyde directly results in dimethylation of the amine since the additional steric hindrance provided by one methyl group is insufficient to prevent the second alkylation. However, other aldehydes do produce exclusively monoalkylated products, as steric hindrance becomes more important than electronic effects. This allows for a multi-step route, where initially a reductive amination with benzaldehyde leads to an easily cleaved benzyl group on the amine. The mono-methylation of this secondary amine by a further reductive amination now occurs cleanly and deprotection yields the final product. This is a relatively short and very mild method which is free of epimerisation (**Scheme 2.7**).



Scheme 2.7 – *N*-methylation of L-threonine and D-threonine

This route was undertaken, initially with L- and D-threonine, from commercial L- and D-threonine methyl ester. Imine formation was carried out by stirring the amino acid with benzaldehyde for one hour, then reduction of the imine with sodium borohydride and acidic workup gave the secondary amines, **2.8** and *ent*-**2.8**, in good yields (93% and 78% respectively). ^{1}H NMR data was in good agreement with the literature report, except that the reported broad singlets at δ_H 3.47 and δ_H 1.59 were not observed. These are likely to be resonances from the exchangeable NH and OH protons and their absence can be explained by their exchange with trace amounts of water present in the solvent ($CDCl_3$) over time. High resolution mass spectral data confirmed the molecular formula of the product.

The next step involved introduction of the methyl group by imine formation with formaldehyde, followed by reduction with sodium cyanoborohydride and acidic workup. Sodium cyanoborohydride, an alternative reducing agent which is milder than the more common sodium borohydride, is commonly used in reductive aminations as it will efficiently reduce an imine but reacts much more slowly with ketones and aldehydes. As this methylation step is carried out without pre-formation of the imine (reductant and acid are added immediately after the aldehyde), this reagent was chosen in order to avoid

consumption of the aldehyde and reductant in the unproductive formation of methanol. The *N*-methylated threonine derivatives, **2.9** and *ent*-**2.9**, were obtained in good yields (80% and 77% respectively). ^{1}H NMR data of the product were in good accordance with those reported, with high resolution mass spectral data confirming the molecular formula. The specific rotation of the product (-98º for **2.9** and +96º for *ent*-**2.9**) was slightly lower than that reported (-112º for **2.9**), but the discrepancy may be due to experimental error rather than being indicative of racemisation.

Cleavage of the benzyl group was carried out cleanly by catalytic hydrogenation using palladium on carbon as a catalyst. The free secondary amines, **2.10** and *ent*-**2.10**, were obtained in moderate to excellent yield (55% and 97% respectively), with ^{1}H NMR data in good agreement with those reported, except for the absence of the exchangeable protons reported. High resolution mass spectral data confirmed the molecular formula of the product. Specific rotation data (-19º for **2.10** and +17º for *ent*-**2.10**) was in good accordance with the reported specific rotation (-18º for **2.10**).

Finally, cleavage of the methyl ester under basic conditions was carried out. Due to the possibility of epimerisation of amino acids under basic conditions, one equivalent of base was employed. The final *N*-methyl amino acids, **2.11** and *ent*-**2.11**, were obtained as lithium salts, in approximately quantitative yield. The ^{1}H NMR data corresponded to those in the literature for the free amine,[42] although some shifts in δ_H were observed. A shift in δ_H is anticipated when comparing unprotected amino acids, as changes in ionization can make significant changes to the electronic environment of the nearby protons.[44] The chemical shift of the protons closest to the ionizable centres are most significantly affected, as can be seen by comparison of the δ_H observed for the α, β and γ protons and those in the literature, given in **Table 2.2**.

Table 2.2 – Changes in chemical shift observed

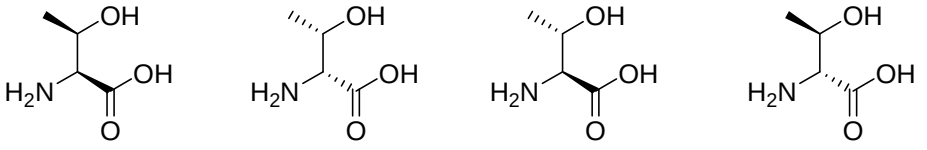
	Observed δ_H	Literature δ_H	$\Delta\delta_H$
α	2.72	3.22	0.5
β	3.68	3.9	0.22
γ	1.05	1.18	0.13
NMe	2.15	2.59	0.44

No optical rotation data are available for the lithium salt of the *N*-methyl-threonines, however the values obtained were moderately similar in value and opposite in sign (-2º for **2.11** and +10º for *ent*-**2.11**). Marfey's derivatisation and HPLC separation confirmed the optical purity of the product (**Figure 2.4**, **Section 2.2.4**).

With *N*-methyl-L- and D-threonine in hand, attention was now turned to the synthesis of *N*-methyl-L- and D-*allo*-threonine.

2.2.4 Attempts to invert the β stereochemistry of N-*methyl-L- and D-threonine*

Of the coded amino acids, isoleucine and threonine are the only examples to contain two stereogenic centres. As a result, they each have four possible diastereoisomers; those of threonine are shown below.



L-threonine (2*S*,3*R*) | D-threonine (2*R*,3*S*) | L-*allo*-threonine (2*S*,3*S*) | D-*allo*-threonine (2*R*,3*R*)

L-threonine is the coded amino acid, is available commercially and is inexpensive. D-threonine is also inexpensive. L- and D-*allo*-threonine, whilst commercially available, are significantly more expensive, with L-*allo*- being around 450 times and D-*allo*- around 850

times as expensive as L-threonine. This makes the synthesis of the *N*-methylated *allo*-stereoisomers significantly more expensive. Whilst the procedure used in **Section 2.2.3** gave a good overall yield of 72%, the "student-induced isotope effect" described by Simpson suggests that this yield will be significantly lower when using more expensive starting materials.[45] An additional esterification step would also be required to give the methyl ester starting material.

This led to the consideration of attempting the inversion of the β position of the *N*-methylated-L- and D-threonines that had been synthesized earlier. This would allow access to the *allo*- diastereoisomers indirectly and at much lower cost.

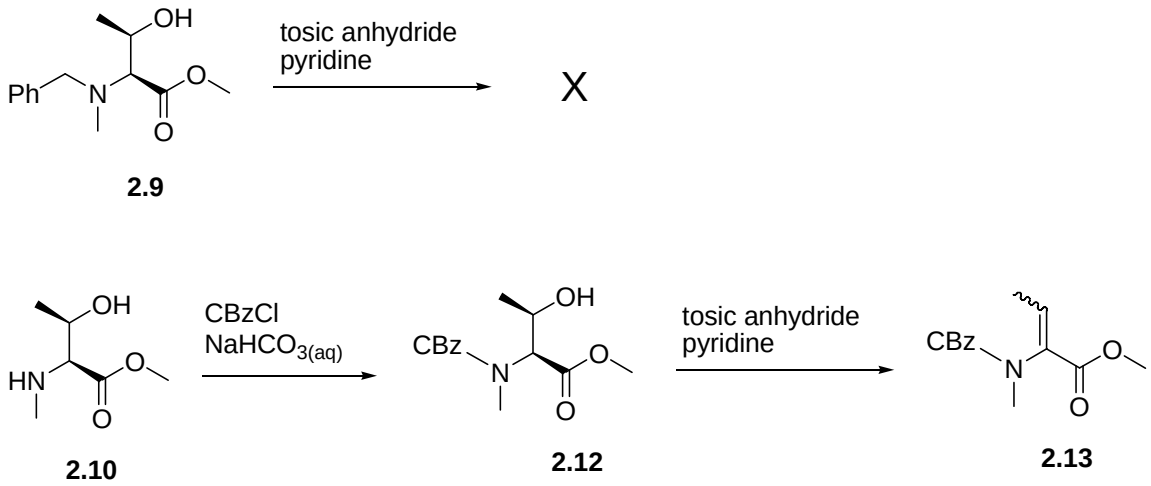
Inversion of the stereochemistry of alcohols is an important procedure which is used regularly in natural product synthesis[46] and in particular in carbohydrate chemistry.[47,48] This is carried out by activation of the alcohol to form a good leaving group, followed by nucleophilic displacement. The traditional means of alcohol activation involves conversion to a sulfonate, eg tosylate or mesylate, which are excellent leaving groups. The nucleophilic displacement can then be carried out either directly with hydroxide, or with alcohol precursors, such as acetate or benzoate which provide protected alcohols. An alternative to the sulfonate route is the widely-used, one-pot, activation-displacement method developed by Mitsunobu *et al*.[49] which will be discussed in more detail later.

Both the tosylate and the Mitsunobu activation routes were investigated as a means of inverting the stereochemistry of the β position.

Initial attempts (**Scheme 2.8**) to invert the stereochemistry at the β position of protected *N*-methyl-L-threonine, **2.9**, involved an attempt to form the tosylate of the alcohol. The reaction was carried out with tosic anhydride in dry pyridine. No desired product could be isolated from the reaction and no other product could be assigned. In case decomposition of the desired product was occurring, the reaction was repeated but was quenched with sodium hydroxide after 5 minutes in the hope of hydrolyzing any tosylate

present. However, only starting material and the product of elimination (discussed below) were recovered from this reaction.

It seemed unlikely that the tertiary amine could be interfering with the formation of the tosylate, however to confirm this, the amine of **2.10** was CBz protected. This was carried out using benzyl chloroformate under Schotten-Baumann conditions and gave **2.12** in a yield of 74%. The tosylation of the carbamate protected *N*-methyl-L-threonine led to the formation of dehydro-*N*-methyl-threonine derivative, **2.13**, and recovery of starting material, with no desired tosylate product isolated. The assignment of the stereochemistry about the double bond of alkene **2.13** was not attempted.



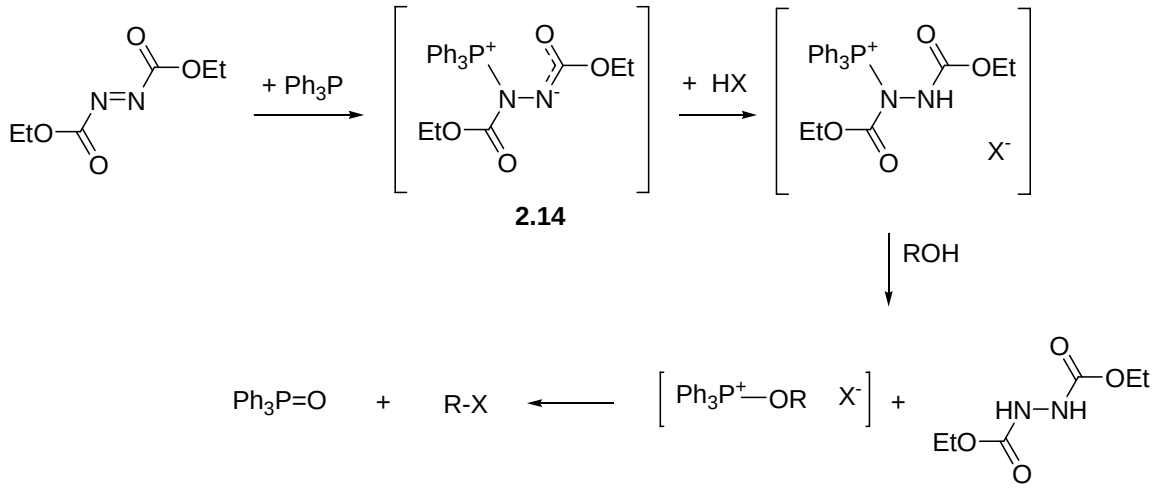
Scheme 2.8 – Tosylation attempts on *N*-methyl-L-threonine derivatives

Elimination can be observed when the proton adjacent to an activated alcohol is acidic; a requirement that is fulfilled by the α proton in an amino acid. The isolation of a dehydration product suggests that the tosyl product was formed, but that it was sufficiently unstable under the basic tosylation conditions to eliminate. A survey of the literature revealed that elimination had also been observed as the main product when tosylation of *N*-Fmoc-threonine benzyl ester had been attempted. The authors noted that varying degrees of tosylation and elimination occurred with different amino and acid protecting groups.[50] They also noted that in the presence of pyridine only (ie no

tosylating agent added) the amino acid derivatives were stable, confirming the intermediacy of an *O*-tosyl group in the elimination step.

It was apparent that a route involving tosylate activation was going to require a great deal of optimisation. It was therefore decided to investigate the Mitsunobu protocol as an alternative method.

As a means of stereochemical inversion of alcohols, the Mitsunobu reaction is undoubtedly one of the most widely-used and studied.[51] It involves the activation of the alcohol as an alkoxyphosphonium salt which is formed by reaction with the betaine, **2.14**, derived from a phosphine (most commonly triphenyl phosphine) and a dialkyl azodicarboxylate (usually diethyl azodicarboxylate). It has been established as a reliably stereoselective reaction, giving inversion of alcohols, and as such, has become popular amongst synthetic chemists for inverting the configuration of optically active alcohols.[52] The mechanism proposed by Mitsunobu is shown in **Scheme 2.9**.



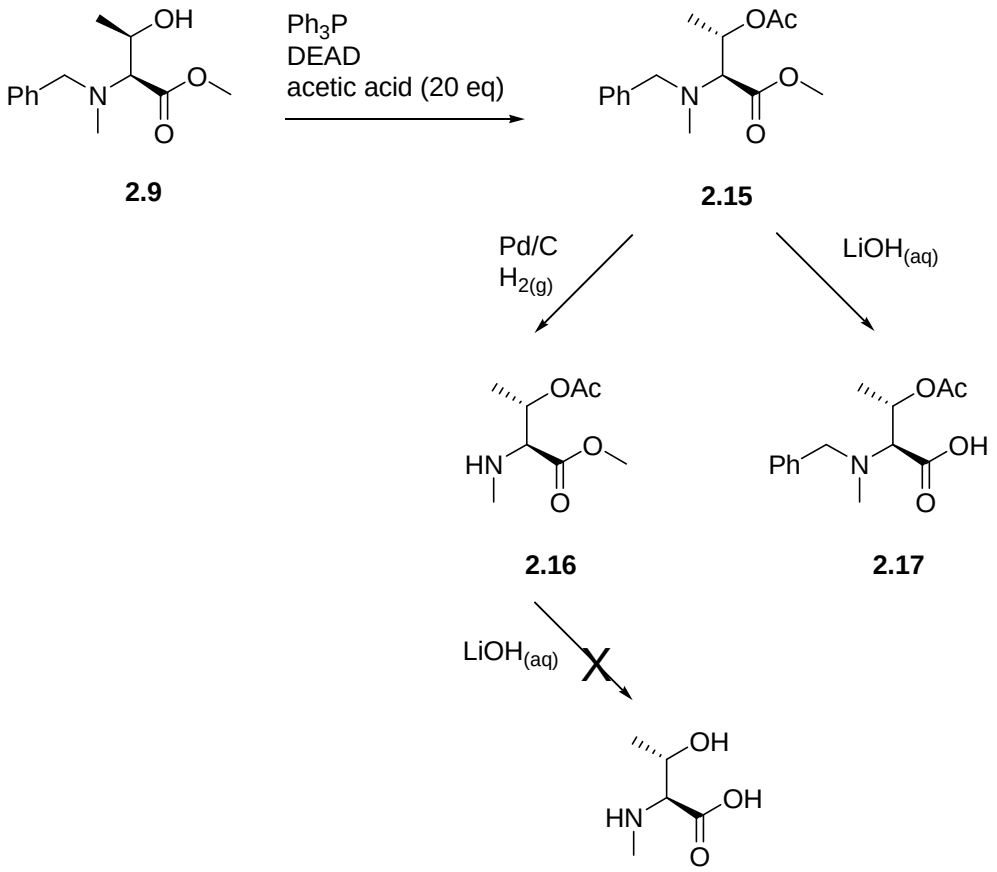
Scheme 2.9 – Reaction mechanism for the Mitsunobu reaction

The Mitsunobu reaction is a redox reaction since during the course of the reaction, the diethyl azodicarboxylate is reduced to a hydrazine, and triphenylphosphine is oxidized to triphenylphosphine oxide. It is the stability of the latter that drives the final nucleophilic displacement of the activated alcohol by the conjugate base X^-. In this scheme the

nucleophile (which must be acidic with pKa<13)[53] has been represented as an ionic acid, and are most commonly carboxylic acids or phenols, however many other acids, both ionic and neutral,[54] can also be used successfully, for example, phthalimide,[55,53] which can then be converted to an amine by hydrazinolysis. The broad range of nucleophiles which can be used to displace the activated alcohol has allowed the Mitsunobu protocol to find application in the introduction of an array of functional groups, and it is a very powerful tool in synthetic chemistry.[49] This brief description of the mechanism of the Mitsunobu reaction will be expanded upon later.

Initial attempts (**Scheme 2.10**) to invert the stereochemistry at the β position of an *N*-methyl-threonine derivative involved the reaction of protected *N*-methylthreonine **2.9** with one equivalent of acetic acid, in the presence of an excess of both triphenylphosphine and diethyl azodicarboxylate. Acetic acid was chosen as the acetate derivative formed could potentially be hydrolysed under the same conditions as the methyl ester. The only product which could be isolated from the reaction was a dehydro-*N*-methyl-threonine derivative (characterized by ^{1}H NMR spectroscopy), formed by elimination of the activated alcohol similar to that observed with tosyl activation. As the most basic species present, the betaine is most likely the vehicle for this elimination reaction.

The reaction was repeated but with a large excess (100 equivalents) of acetic acid, in an attempt to quench the alkyloxyphosphonium salt before elimination occurred, and ensure that the betaine deprotonated the nucleophile instead of the activated alcohol. This reaction gave some of the desired acetate, **2.15**, (8%) but largely starting material was recovered (84%). It is postulated that the increased acidity of the reaction mixture under these conditions may be preventing the formation of the alkyloxyphosphonium salt, so therefore the reaction was repeated with fewer equivalents (20) of acetic acid. Under these conditions a respectable yield of 51% was obtained, accompanied by good recovery of starting material (48%).



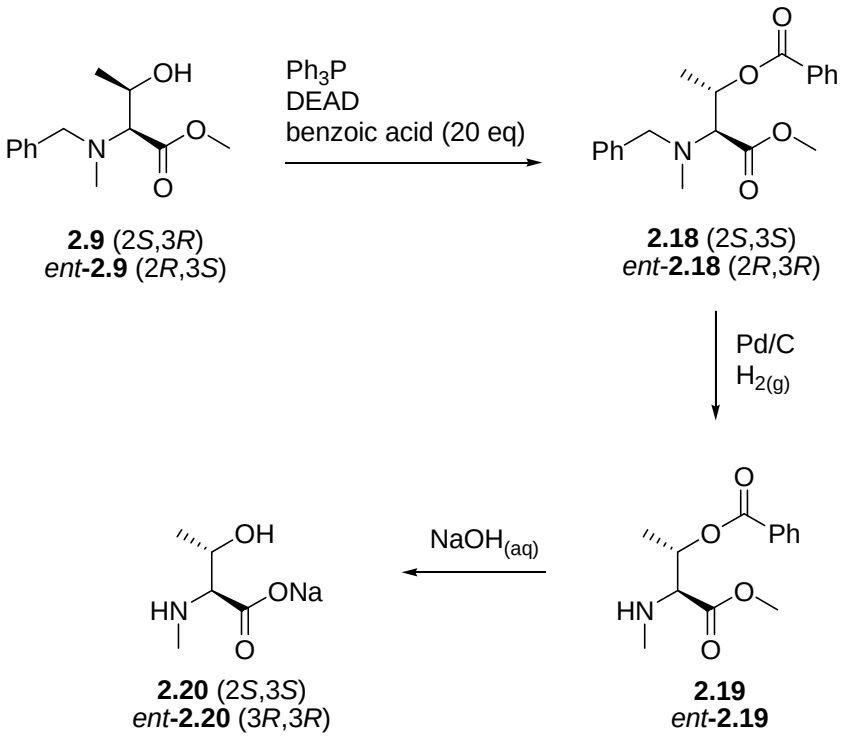
Scheme 2.10 – Mitsunobu inversion and deprotection attempts

With the inverted acetate, **2.15**, in-hand, deprotection was attempted to give the *N*-methyl-*allo*-L-threonine. The same hydrogenation conditions were employed as those used to deprotect **2.9** and *ent*-**2.9** (**Section 2.2.3**). The crude product of the reaction appeared to be the correct product, **2.16**, however the product was quite impure. Purification by successive phase separations did not significantly improve the purity. The crude product was therefore hydrolysed using lithium hydroxide, but the ^{1}H NMR spectrum of the product was complicated and appeared still to contain signals indicative of an acetate group. By inverting the two deprotection steps it was hoped that these problems might be circumvented. Therefore the hydrolysis of acetate **2.15** was attempted using lithium hydroxide. One clean product was obtained with 3 equivalents of base, however the product clearly still contained an acetate group. It was clear that cleavage of the acetate group would require more forcing basic conditions, which could cause epimerisation of the α proton. Acidic conditions can also be used to cleave ester groups,

however, high temperatures are commonly required; conditions which can cause elimination of the alcohol in threonine derivatives.

The use of an alternative nucleophilic acid in the Mitsunobu reaction, was considered as a possible solution to the deprotection problems discussed above. Benzoic acid is a very commonly used nucleophile in Mitsunobu reactions, and whilst a benzoate would be expected to be slightly more difficult to hydrolyse than an acetate, it was chosen as an alternative nucleophile.

The Mitsunobu reaction was therefore attempted on the protected *N*-methyl-D-threonine, *ent*-**2.9**, (the D derivative was used as more was available) using 20 equivalents of benzoic acid (**Scheme 2.11**). This, pleasingly, gave an excellent yield of 95% of *ent*-**2.18** if the reaction was allowed to proceed for three days. The deprotection of *ent*-**2.18** began with a catalytic hydrogenation, which on this occasion proceeded smoothly to give the clean product, amine *ent*-**2.19**, in 76% yield. The final hydrolysis step was carried out, on this occasion, using sodium hydroxide. This led to the complete hydrolysis of both the benzoate and the methyl ester, giving a quantitative yield of the *allo*-*N*-methyl-amino acid *ent*-**2.20** as a sodium salt. The hydrolysis was also attempted using lithium hydroxide, as had been used for deprotection of the acetate derivative, **2.15**, discussed earlier. The product obtained under these conditions displayed a complicated mixture by ^{1}H NMR spectroscopy, suggesting that the problems in deprotection encountered may have been due to the use of the inappropriate base. The deprotection of acetate **2.15** was not revisited, however.



2.9 (2*S*,3*R*)
ent-**2.9** (2*R*,3*S*)

2.18 (2*S*,3*S*)
ent-**2.18** (2*R*,3*R*)

2.20 (2*S*,3*S*)
ent-**2.20** (3*R*,3*R*)

2.19
ent-**2.19**

Scheme 2.11 – Mitsunobu reaction and successful deprotection

Since this route had proved successful, it was repeated with **2.9** to give **2.20**, in similar yields. With *N*-methyl-threonine of both L-*allo* and D-*allo* configuration in hand, the synthesis of all four stereoisomers of *N*-methyl-threonine was deemed complete. Examination of the optical purity of the four stereoisomers was carried out by derivatisation of the amino acids with Marfey's reagent and separation by HPLC chromatography. The results are shown in **Figure 2.4**.

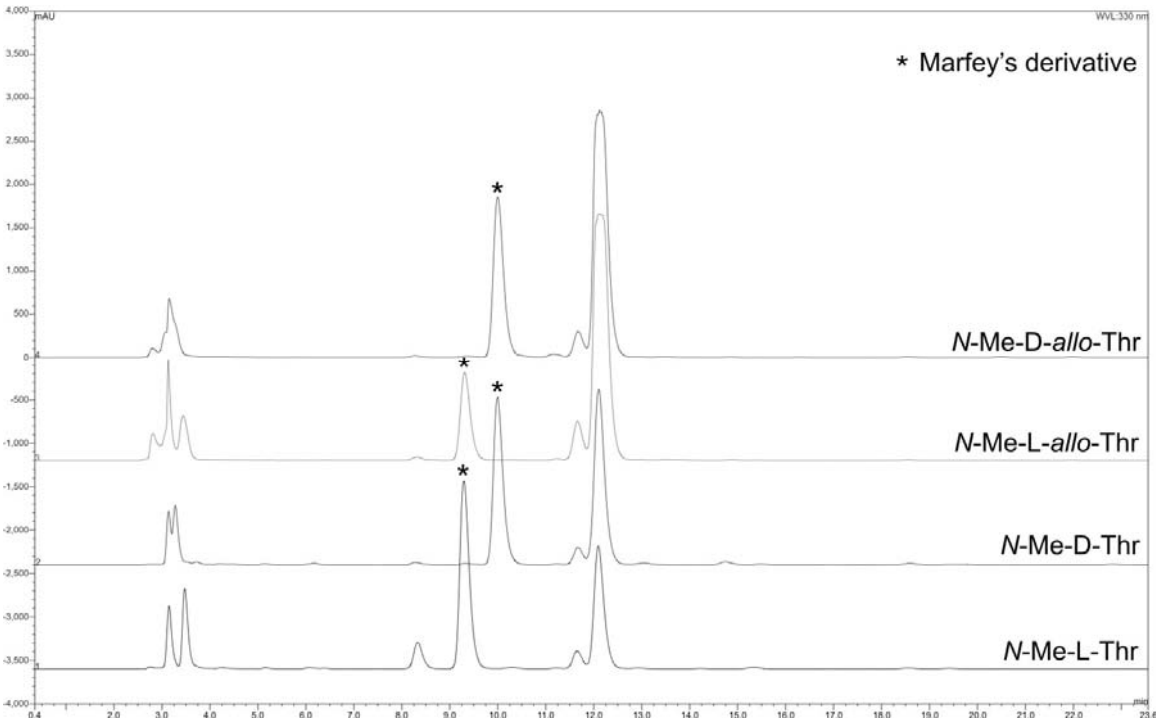


Figure 2.4 – Marfey's derivatives of the stereoisomers of *N*-methyl-threonine

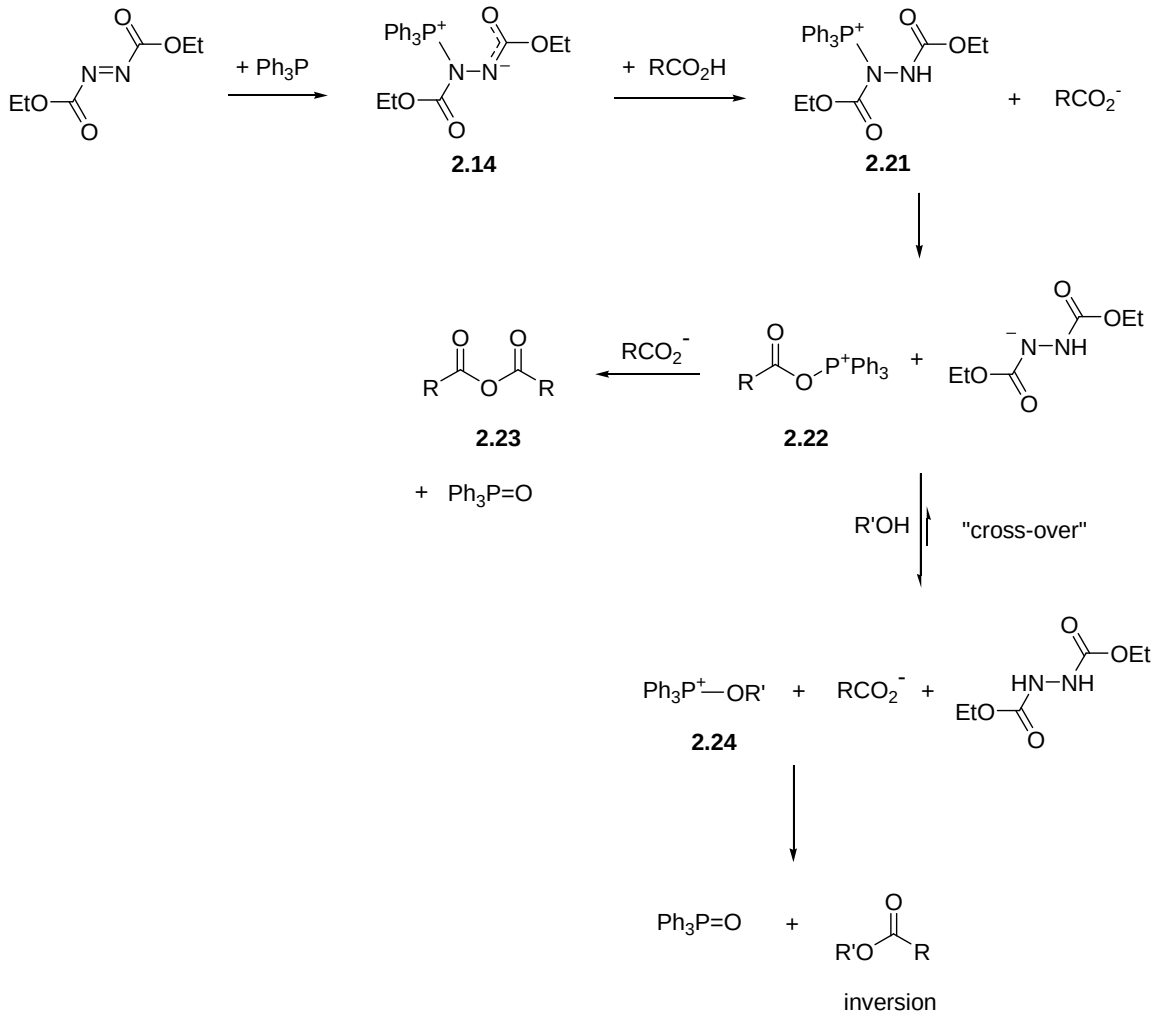
These results were surprising, in that no separation of L (**2.11**) from L-*allo* (**2.20**) was observed, and likewise with D (*ent*-**2.11**) from D-*allo* (*ent*-**2.20**). It would be expected that there is a sufficiently significant difference in the physical properties of the *allo*-series from the natural series to allow separation by HPLC. A series of HPLC methods were attempted in an effort to achieve separation. In each method, different parameters were explored; both methanol and acetonitrile were used as eluents, the column temperature was altered, and isocratic and gradient methods were employed. A method described in the literature for separating the four stereoisomers of *N*-methyl-thronine was also attempted.[56] However, under all of these varied conditions, the L and L-*allo* pair invariably had the same retention time, and likewise for the D and D-*allo* pair.

It became clear that the problem was not a separation issue, but rather that the Mitsunobu reaction had not occurred with the anticipated inversion of stereochemistry and had instead occurred with complete retention. Therefore **2.20** was actually **2.11** and *ent*-**2.20** was *ent*-**2.11**. This is very surprising in light of this reaction's position as the "industry standard" for inversion of alcohols and its well-documented reliability. A study of recent

literature, however, showed that retention in the Mitsunobu reaction has been documented, albeit rarely. A number of independent studies reported the isolation of retention products from sterically hindered chiral secondary alcohols when using carboxylic acids as the nucleophile. As a result, the accepted mechanism of the Mitsunobu reaction has come under close scrutiny.[52] Some fascinating new insights have been made in the last few years which have begun to uncover subtle but important mechanistic details, unrecognised by Mitsunobu. These are still under investigation, but some of these mechanistic modifications are described below (**Section 2.2.5**).

2.2.5 The mechanism of the Mitsunobu reaction

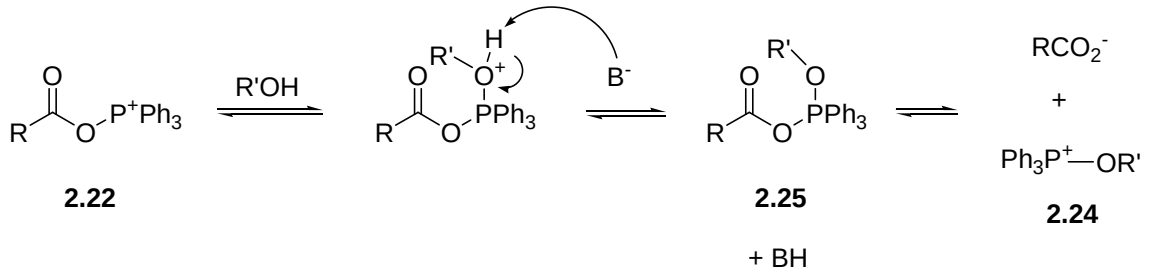
The most important factor which alters the stereochemical outcome of the Mitsunobu reaction is the use of sterically hindered substrates.[57,58] The electronic nature of the phosphine used,[59] the presence of a base,[60] conformational constraints[61] and substrate-specific rearrangements of reaction intermediates[62] have also been implicated. These have been rationalised largely through the inclusion of an important intermediate during the course of the Mitsunobu reaction which had not been postulated previously. This is the acyloxyphosphonium salt, **2.22**, and the anhydride, **2.23**, which can be formed from **2.22** with a second equivalent of carboxylic acid. A revised mechanism is portrayed in **Scheme 2.12**.



Scheme 2.12 – Revised Mitsunobu mechanism

In the originally proposed mechanism, after protonation of the betaine by the carboxylic acid, the alcohol attacks the phosphorus centre of **2.21** giving an alkyloxyphosphonium salt, **2.24**, directly. This intermediate is then attacked by the nucleophilic carboxylate anion with inversion to give the ester product. The major difference in the newly proposed mechanism is that it is the more nucleophilic carboxylate anion which attacks the betaine, generating an acyloxyphosphonium salt, **2.22**, and a hydrazide anion. This initially formed intermediate then undergoes a base-mediated cross-over reaction to the alkyloxyphosphonium salt, **2.24**, via an acyloxyalkoxyphosphorane, **2.25** (**Scheme 2.13**).[60] The base would usually be the hydrazide anion. Under normal Mitsunobu

conditions and with relatively unhindered substrates, this equilibrium lies towards **2.24**, and attack by the carboxylate anion generates the ester with inversion of stereochemistry.



Scheme 2.13 – Base-mediated "cross-over" reaction

A number of factors can shift this equilibrium in favour of **2.22**, however, and the most important of these is steric hindrance in the alcohol. This inhibits the formation of **2.25** and therefore prevents the "cross-over" to **2.24**. The long lifetime of **2.22** allows alternative pathways to compete effectively. Acyloxyphosphonium salt **2.22** is an activated acid, and as such can undergo esterification by attack of an alcohol at the carbonyl centre with displacement of triphenylphosphine oxide. This reaction does not involve inversion at the alcohol and therefore generates an ester with retention of stereochemistry at this centre. Under some conditions, **2.22** may react with a further carboxylate anion to form an anhydride, **2.23**.[63] The anhydride is also an activated acid and may also form an ester with retention of stereochemistry.

Indirect evidence for the involvement of an acyloxyphosphonium salt was described by McNulty *et al*. by generating the salt by an alternative method, and reacting it with L-menthol.[60] The ester formed was present in 97% as the product of retention (3% inversion). In this fascinating paper, they also investigated the role of base in the "cross-over" step. By including an amine base in the reaction they found that the stereochemical outcome could be reversed, with up to 99% of the inversion ester produced instead.

Taking into account this new mechanism, it is possible to postulate why the ester obtained from Mitsunobu reaction with *N*-methyl-*N*-benzyl-threonine methyl ester, was the product of retention of stereochemistry and not the intended inversion product **2.18**.

In order to suppress competing elimination reactions, the number of equivalents of benzoic acid used in the reaction was greater than that usually employed. It is probable that this excessively acidic medium quenched any basic species, such as the betaine or hydrazide anion, before the base-mediated conversion of acyloxyphosphonium salt, **2.22**, to alkoxyphosphonium salt, **2.24**, could occur. The activated acid was then available for attack by the alcohol of the precursor, **2.9**, giving the ester with retention of stereochemistry.

Despite the new information available on the mechanism of the Mitsunobu reaction, one question remains unanswered. In the majority of cases where the inversion reaction is disfavoured, a low yield or no reaction is observed. However, in this small number of examples moderate to good yields with complete retention are observed. In many of these cases sterically hindered alcohols are involved; seemingly disfavouring the nucleophilic attack of the alcohol on the carbonyl of the acyloxyphosphonium salt. It is interesting to speculate why retention is observed in certain cases. One common theme amongst several reports[58,61,62] observing retention products (although not exclusively)[57] is that they are using the Mitsunobu reaction to achieve intramolecular lactonisation. It may be that by having the acyloxyphosphonium salt in the same molecule as an alcohol promotes the esterification sufficiently to observe the retention product. There appears to be an active research field currently investigating aspects of the mechanism of the Mitsunobu reaction and hopefully this will, in the future, uncover further details.

A number of amendments were made to the conditions used to carry out the Mitsunobu reaction on *N*-methyl-*N*-benzyl-L-threonine methyl ester in an attempt to promote the inversion reaction.

As mentioned earlier, the addition of base has been shown to efficiently promote the "cross-over" step which leads to inversion. Mitsunobu reactions with the addition of an excess of ^{t}butylamine, pyridine and triethylamine were therefore all attempted, but in each case only starting material was recovered. Base, therefore, is able to prevent the

retention pathway from occurring, but does not promote the inversion reaction, in this case.

The use of a salt of benzoate instead of the acid was considered as a means of avoiding the acidic conditions. It has been reported that the choice of nucleophile counter-ion in Mitsunobu reactions is of significant importance, with tight ion-pairing preventing reaction.[64,65] Zinc salts of nucleophiles are reported to react efficiently under Mitsunobu reaction conditions, so the zinc salt of benzoic acid was formed by following literature procedure for the similar zinc *p*-chlorobenzoate salt.[66] The Mitsunobu reaction, again, did not take place, and only starting materials were recovered.

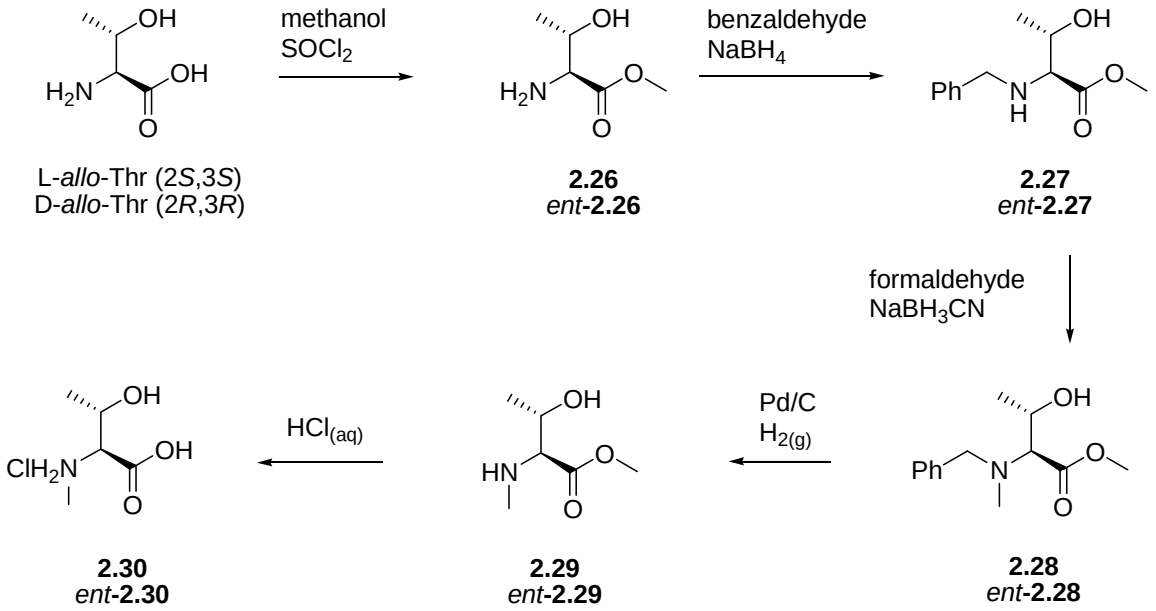
A reaction was carried out with a large excess of triphenylphosphine and diethyl azodicarboxylate (11 equivalents each and 10 equivalents of benzoic acid), in the hope that sufficient betaine would remain after reaction with benzoic acid to act as a base. Starting materials were, again, the only product isolated from this reaction.

Whilst benzoic acid has been the most popular choice of carboxyl nucleophile in Mitsunobu esterification reactions, it has recently been shown that it is not necessarily the most appropriate. The pK_a of the carboxylic acid used as the nucleophile is known to have a profound influence on the product yield of Mitsunobu reactions when sterically hindered alcohols are employed.[67] The use of *p*-nitrobenzoic acid (pK_a 3.41)[68] and chloroacetic acid (pK_a 2.85)[69] were reported to give improved yields compared to benzoic acid (pK_a 4.15). It has been shown that this is due to a shift in the equilibrium between the acyloxy- and alkoxy-phosphonium salt.[70,63] A lower limit is placed on the pK_a of the nucleophile, however, as the acid must still be sufficiently nucleophilic to attack the alkoxyphosphonium salt. Attempts were made to couple the threonine precursor to both *p*-nitrobenzoic acid and chloroacetic acid. The major product of the Mitsunobu reaction using *p*-nitrobenzoic acid was the dehydration product, with small amounts of starting material recovered, but no desired product was formed. In the case of chloroacetic acid only starting material was recovered.

It became clear that the inversion of the β centre of *N*-methyl-threonine derivatives gave rise to significantly more complex results than anticipated and it was decided to cease attempts to access the *allo*- series in this way. A large number of variables had been explored in the Mitsunobu reaction, but to no avail. This project gave some interesting results which could only be accounted for by achieving a deeper understanding of the subtleties of the Mitsunobu reaction mechanism.

2.2.6 Synthesis of N-methyl-L-allo- and D-allo-threonine

As inversion of the β centre of *N*-methyl-threonine derivatives had not been achieved, the synthesis of the *allo*- series was carried out using the original method discussed above (**Section 2.2.3**), using the expensive, commercially available L-*allo*- and D-*allo*-threonine as starting materials (**Scheme 2.14**). An additional step was required, to form the methyl ester from the free amino acids. This was carried out by the classical method of refluxing the amino acid in methanol with thionyl chloride and the methyl esters **2.26** and *ent*-**2.26** were obtained in quantitative yield (characterized by ^{1}H NMR spectroscopy).



L-*allo*-Thr (2*S*,3*S*)
D-*allo*-Thr (2*R*,3*R*)

2.26
ent-**2.26**

2.27
ent-**2.27**

2.28
ent-**2.28**

2.29
ent-**2.29**

2.30
ent-**2.30**

Scheme 2.14 – Synthesis of L- and D-*allo*-*N*-methyl-threonine

The first reductive amination, to introduce the benzyl protecting group, proceeded smoothly as before to give the secondary amines **2.27** and *ent*-**2.27** in 60 and 62% yield respectively. The ^{1}H NMR data of the *allo*- products displayed some differences to the natural series, as anticipated. The largest chemical shift differences were at the α and β centres ($\Delta\delta_H$ of both centres was 0.38). Optical rotation data are not available in the literature for these compounds, however they were of similar value, but opposite sign (-56º for **2.27** and +50º for *ent*-**2.27**), as expected.

The second reductive amination to introduce the methyl group also proceeded cleanly giving protected *N*-methylthreonines **2.28** and *ent*-**2.28** in yields of 88 and 95% respectively. The ^{1}H NMR data were, again, in good agreement with the natural series, with minor changes in chemical shift. In this case the β ($\Delta\delta_H$ of 0.19) and γ ($\Delta\delta_H$ of 0.13) centres displayed the greatest variation. Again, optical rotation data are not available in the literature, but they were of similar value and opposite in sign (-128º for **2.28** and +129º for *ent*-**2.28**).

Deprotection of the benzyl group by catalytic hydrogenation proceeded in moderate yields of 80 (**2.29**) and 56% (*ent*-**2.29**). The ^{1}H NMR data were in good agreement with the natural series, although again, changes in chemical shifts were observed. The main differences were at the α ($\Delta\delta_H$ of 0.35) and β centre ($\Delta\delta_H$ of 0.38). Optical rotation data (also not available from the literature) for each were of similar value and opposite in sign (-4º for **2.29** and +4º for *ent*-**2.29**).

Finally, deprotection of the methyl ester by acidic hydrolysis proceeded cleanly in quantitative yield to give the *N*-methylated amino acids **2.30** and *ent*-**2.30** as hydrochloride salts. The ^{1}H NMR data compared well to the natural series, but with some large changes in chemical shifts (eg $\Delta\delta_H$ of 1.14 for α and $\Delta\delta_H$ of 0.62 for the β). Throughout the synthesis of the *allo*- series, the coupling constants of the α proton were found to be somewhat different (> 5 Hz) from those observed in the natural series. An optical rotation of +6º was obtained for **2.30**, however insufficient material was obtained

to obtain a reliable optical rotation value for *ent*-**2.30**. Marfey's derivatisation and HPLC analysis (below) allowed the purity of the isomers to be confirmed, however.

With the *allo*- series now in hand, Marfey's derivatisation and HPLC separation of the four stereoisomers was repeated (**Figure 2.5**). All four stereoisomers were readily separable on C_{18}. In both the L- and D-pair (**2.11** and *ent*-**2.11**), and the L-*allo*- and D-*allo*- pair (**2.30** and *ent*-**2.30**), the L- isomer eluted before the D-; following the reported trend for Marfey's derivatives.

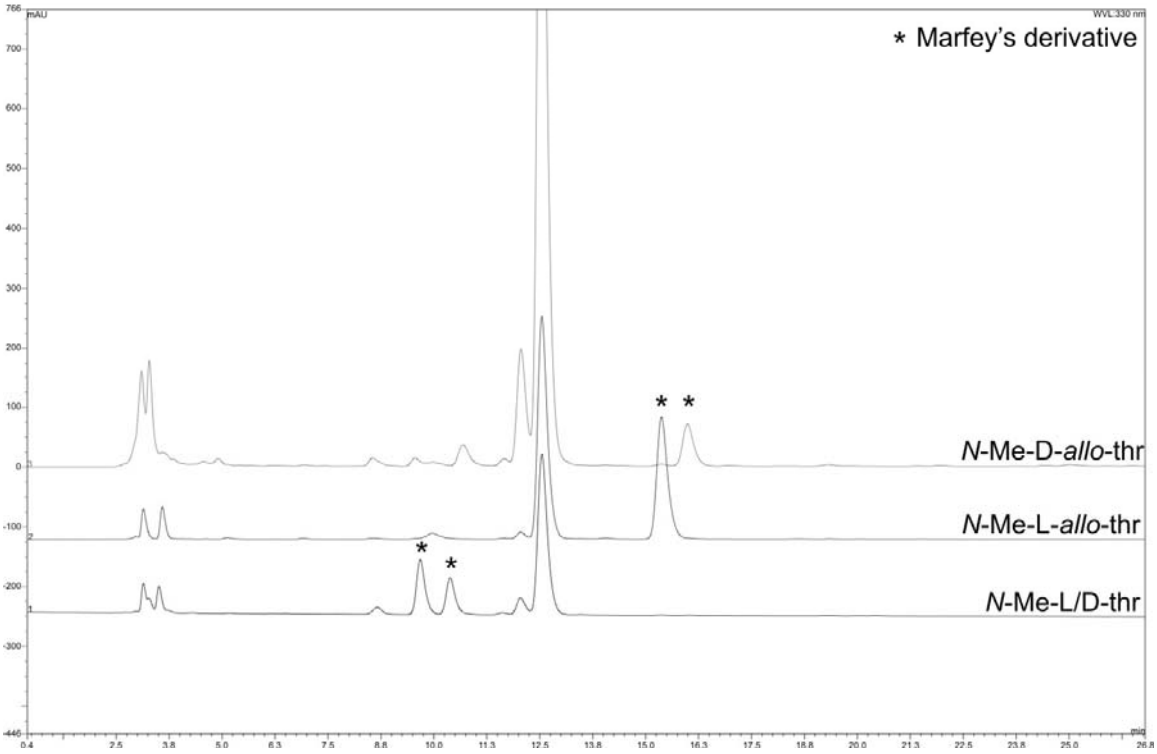


Figure 2.5 – HPLC of the Marfey's derivatives of the four stereoisomers of *N*-methyl-threonine

2.3 Synthesis of 4-methylproline

2.3.1 Introduction

One of the most interesting residues contained in the pteratide series is 4-methylproline, found in pteratides I and II. This is a rarely encountered amino acid, however it has been found from a number of different sources, as discussed in **Section 2.1.2**. A number of other alkyl-proline derivatives are also known; from natural sources[71,72,73] and synthetic analogues.[71] In recent years they have been successfully utilised in the drug development of novel angiotensin converting enzyme inhibitors such as Fosinopril (trade name Monopril).[74]

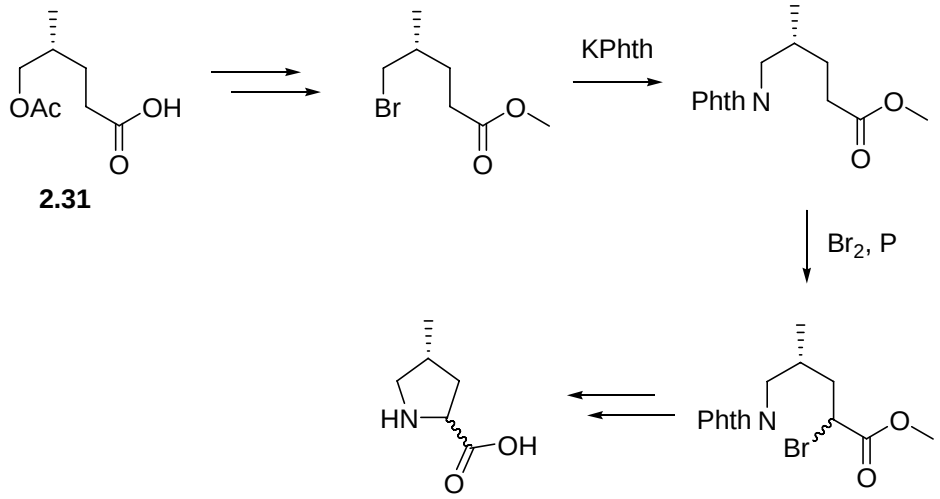
The inclusion of such a small and inert group on an otherwise very common amino acid might suggest that the methyl group of 4-methylproline has little role to play in the bioactivity of the pteratides. However, an organism has to invest a great deal of evolutionary energy in developing the biosynthetic pathways to unusual amino acids, so that it is unlikely that the change plays no role. Since a methyl group cannot be involved directly in bonding (covalent, hydrogen, electrostatic or π-stacking) it is most likely to be involved in altering the conformation adopted by the depsipeptide, promoting the adoption of a bioactive conformer. Proline residues are commonly found in peptides and proteins due to their ability to induce twists in the amide backbone with subsequent effects on secondary structure. It may be that the introduction of a methyl group on the proline induces further conformational changes to the backbone; by altering the conformation of the proline itself, or by steric interactions with other residues within the depsipeptide.

In order to further investigate the effects of this unusual amino acid in the bioactivity of the pteratides, it is important to know its stereochemistry in the natural products. This would allow more informed prediction of the conformational effects this residue may be inducing in the backbone of the depsipeptide. It was therefore important to obtain all four stereoisomers of 4-methylproline for comparison to the natural product hydrolysate

to establish which was present. The synthesis of 4-methylproline has been carried out previously by a number of different routes, by several authors. These are briefly reviewed here.

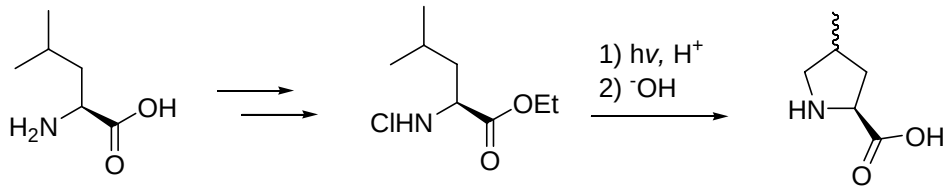
The synthetic routes to 4-methylproline almost all involve manipulation of common amino acid derivatives, as these are commercially available and can be obtained in optically pure form at the α carbon. As a result, only one new stereogenic centre must be generated during the reaction sequence, if epimerisation at the α centre is avoided. The different methods offer varying degrees of stereoselectivity during the introduction of the second stereogenic centre at the γ position.

The earliest literature synthesis of 4-methylproline, published in 1962 by Dalby *et al*., is the only example which begins with the eventual γ position stereogenic centre defined.[75] It uses the industrial by-product, **2.31**, as a starting point and involves cyclisation by displacement of a bromine α to the carboxylic acid by an amine to generate the amino acid moiety (**Scheme 2.15**). After ring-closing, the diastereoisomers could be separated by crystallisation or by enzymatic processes, however these separations were poor yielding and difficult.



Scheme 2.15 – Dalby's synthesis of 4-methylproline

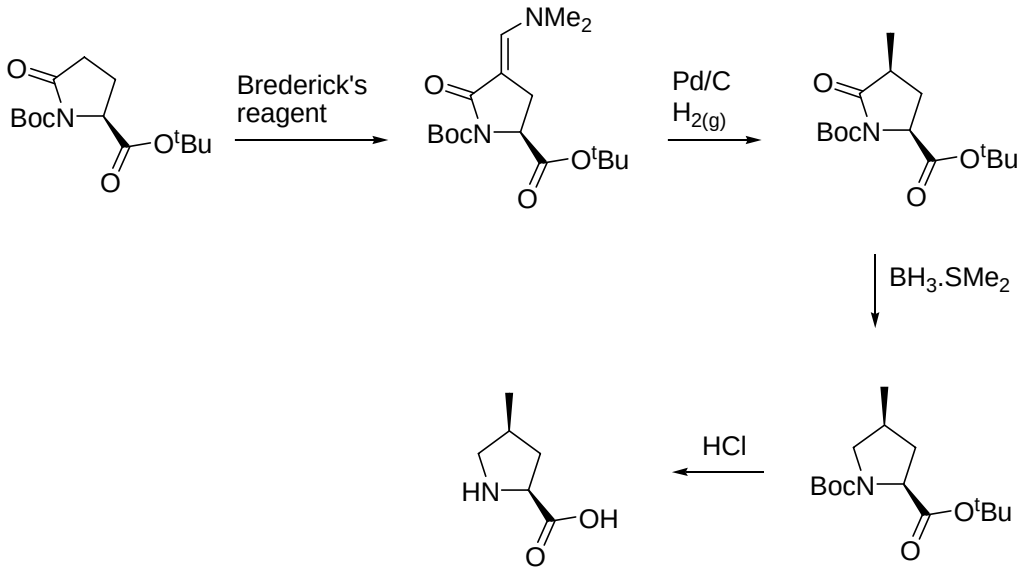
A radical cyclisation (Hofmann-Loeffler-Freytag reaction) of *N*-chloro-L-leucine was reported to give a diastereomeric mixture of *cis*- and *trans*-4-methyl-L-proline (**Scheme 2.16**).[76] Separation of the stereoisomers was achieved in a similar manner to Dalby *et al*.



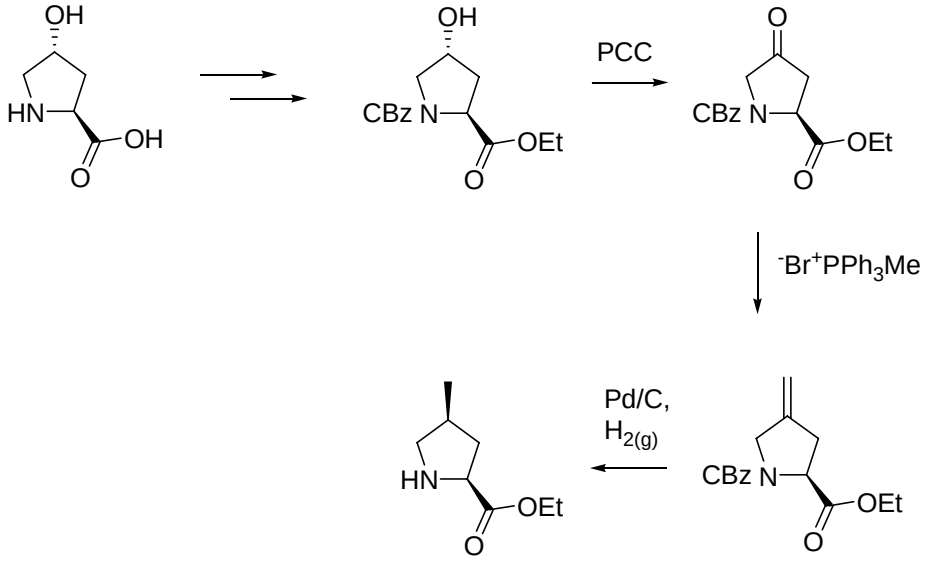
Scheme 2.16 – Titouani's route to 4-methylproline

To avoid the tedious separation issues associated with these routes, stereoselective syntheses began to emerge. These relied on asymmetric induction methods, whereby the chirality of the centre to be formed is influenced by other stereogenic centres already present in the precursor. As mentioned earlier, generally the optically pure amino acid precursors provided the template for this chiral induction. The most popular precursors were pyroglutamic acid and 4-hydroxyproline, both of which are commercially available.

Krapcho's synthesis of *cis*-4-phenylproline and *cis*-4-cyclohexylproline from 4-hydroxyproline,[74] Moody's synthesis of *cis*-4-methylproline from pyroglutamic acid[77,78] and Chirgadze's synthesis[79] of *cis*-4-methylproline from 4-hydroxyproline all utilised a facially-selective catalytic hydrogenation to induce *cis*- stereoselectivity at the γ position (**Scheme 2.17** and **Scheme 2.18**). This idea was extended by Del Valle (**Scheme 2.22**, *vide infra*).

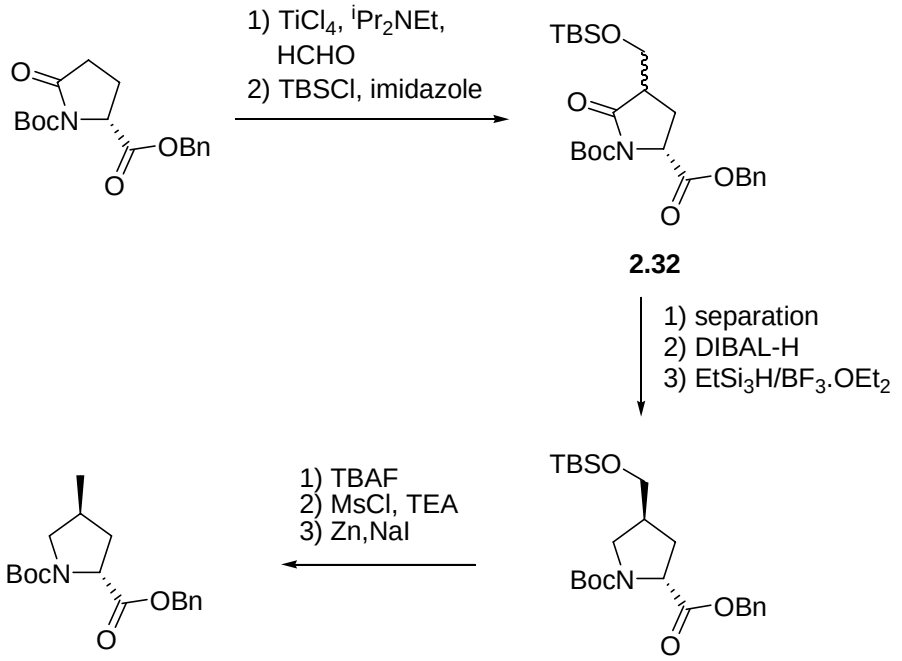


Scheme 2.17 – Moody's *cis*-4-methylproline synthesis



Scheme 2.18– Chirgadze's *cis*-4-methylproline synthesis

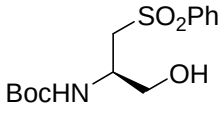
The condensation of formaldehyde with pyroglutamic acid and subsequent manipulations reported by Xie *et al*. gave protected 4-methylproline with a small preference (1.7:1) for the *trans*- diastereoisomer (**Scheme 2.19**).[80] The two diastereoisomers of **2.32** were readily separable using chromatographic methods.



Scheme 2.19 – Xie's synthetic route to protected 4-methylproline

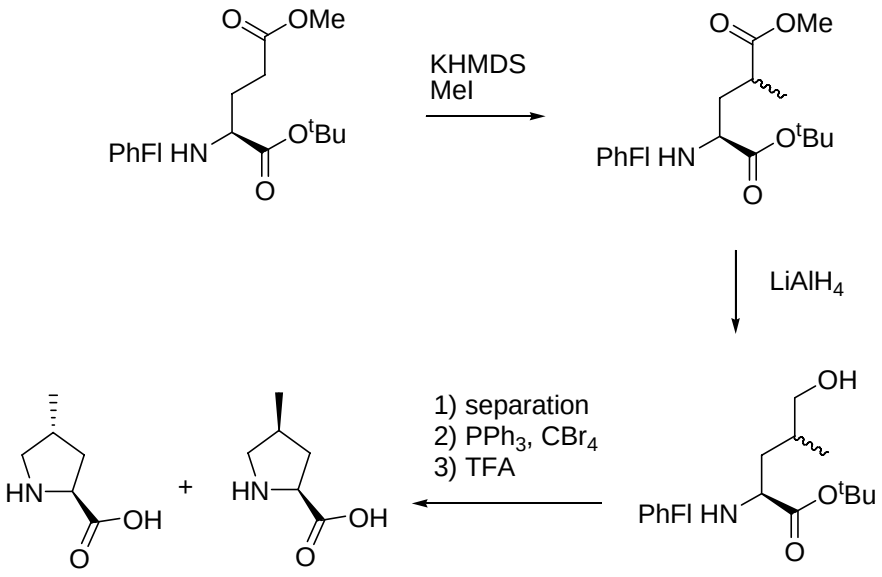
Belokon *et al*. described the use of a chiral Nickel complex template to induce limited diastereoselectivity at the γ position during the condensation of α-methylacrylaldehyde with glycine, giving 4-methylproline.[81]

Wang and co-workers reported a very long, but stereoselective synthesis of *trans*-4-methylproline via the chiral synthon **2.33**.[82]



2.33

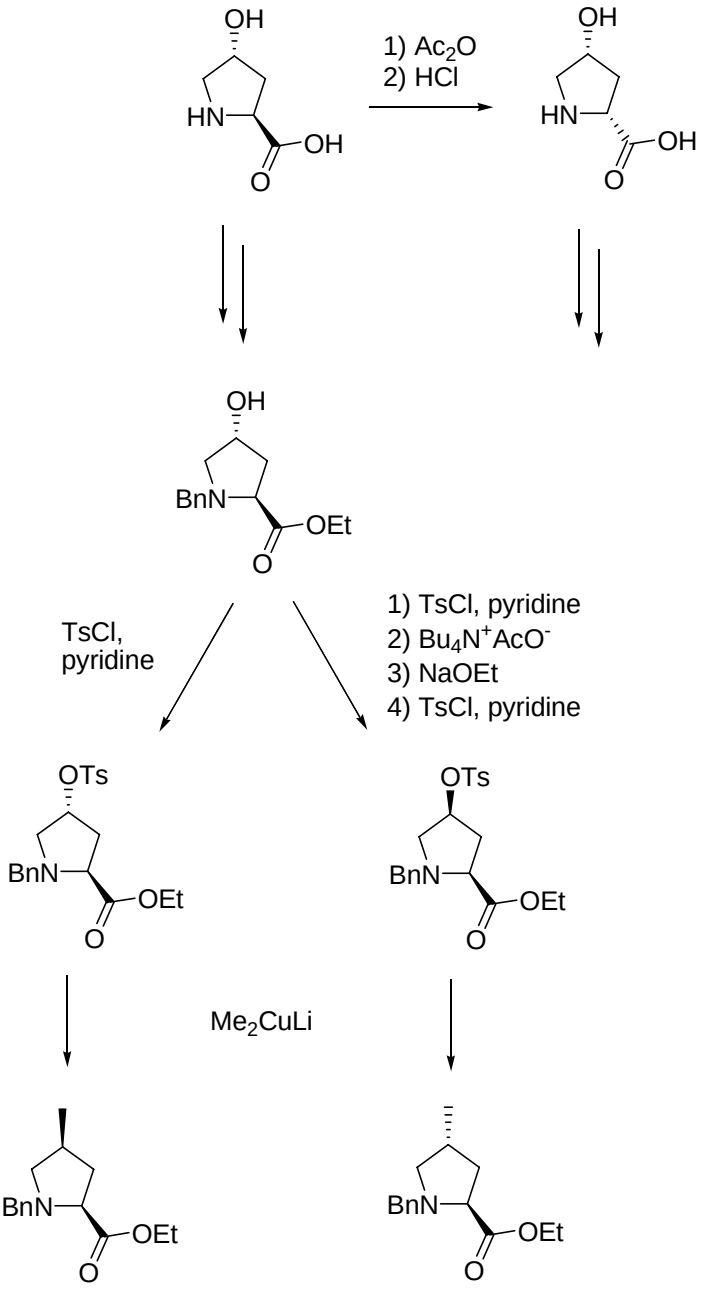
The alkylation of a protected glutamic acid derivative was used by Koskinen and Rapoport to make a number of 4-alkylprolines and displayed a slight stereoselectivity towards forming the *cis*- diastereoisomer (ratio of 2:1 observed for the synthesis of 4-methylproline). [83] Separation of the isomers could be achieved using chromatographic methods (**Scheme 2.20**).



Scheme 2.20 – Koskinen's route to 4-methylproline

These routes all have significant drawbacks, with most displaying poor or no stereoselectivity, resulting in the need to separate isomers. Those routes which do display good stereoselectivity result in the synthesis of only one diastereoisomer. There was clearly a need for routes which would allow reliable stereospecific access to each diastereoisomer of 4-methylproline from a common starting material, and two interesting methods appeared in 2003.

The first of these is Heindl and co-workers' report of the stereoselective synthesis of protected 4-methylproline derivatives from 4-hydroxyproline.[84,85] In this route (**Scheme 2.21**) the alcohol of a protected 4-hydroxyproline is activated by formation of a tosylate group which is subsequently displaced using a methyl nucleophile. The Gilman cuprate reagent was used as methyl nucleophile as it is a mild nucleophile which helps to minimise competing methylation at the carbonyl group of the protected acid.

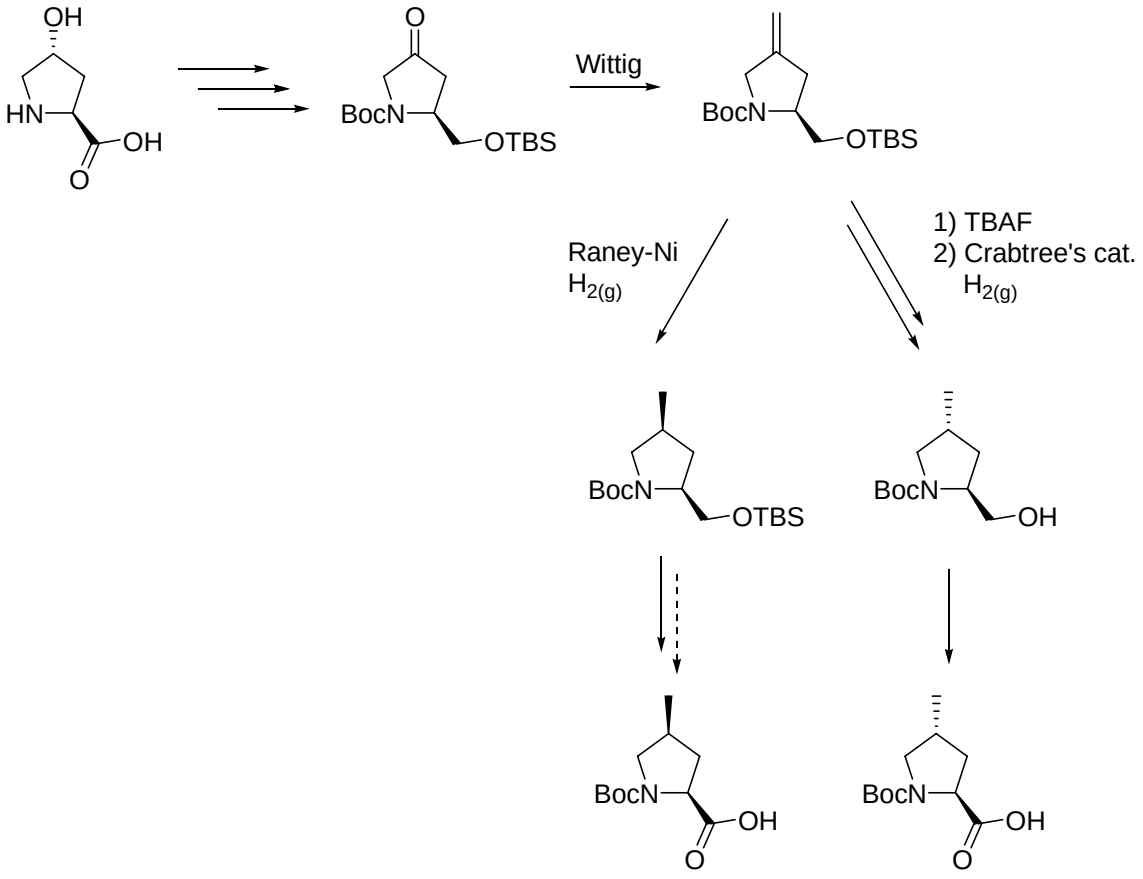


Scheme 2.21 – Heindl's stereospecific synthesis of 4-methylproline

This route allows access to all four diastereoisomers of 4-methylproline from a single, commercially available diastereoisomer of 4-hydroxyproline. As such, it is a powerful new method. However, it does suffer a very low yield during methylation to generate the *cis*- series (11%; 70% was observed in the *trans*- series). This can be attributed to

additional steric hindrance slowing nucleophilic displacement, allowing side-reactions to dominate.

The other stereoselective method to 4-alkyl-prolines was reported by Del Valle and Goodman,[86] who used asymmetric hydrogenation procedures to induce stereoselectivity (**Scheme 2.22**). They extended the scope of the previous examples discussed (*vide supra*). In those cases, steric bulk induces facial selectivity during hydrogenation of either an exocyclic[78] or endocyclic[74] double bond, giving good stereoselectivity for the *cis*- diastereoisomer. Del Valle and Goodman took advantage of the development of Crabtree's homogeneous hydrogenation catalyst,[87] which can give excellent stereoselective control when a group capable of coordinating to a metal (eg hydroxyl) which can direct the hydrogenation is present.[88] In this way they were able to access, stereoselectively, the *trans*- diastereoisomer.



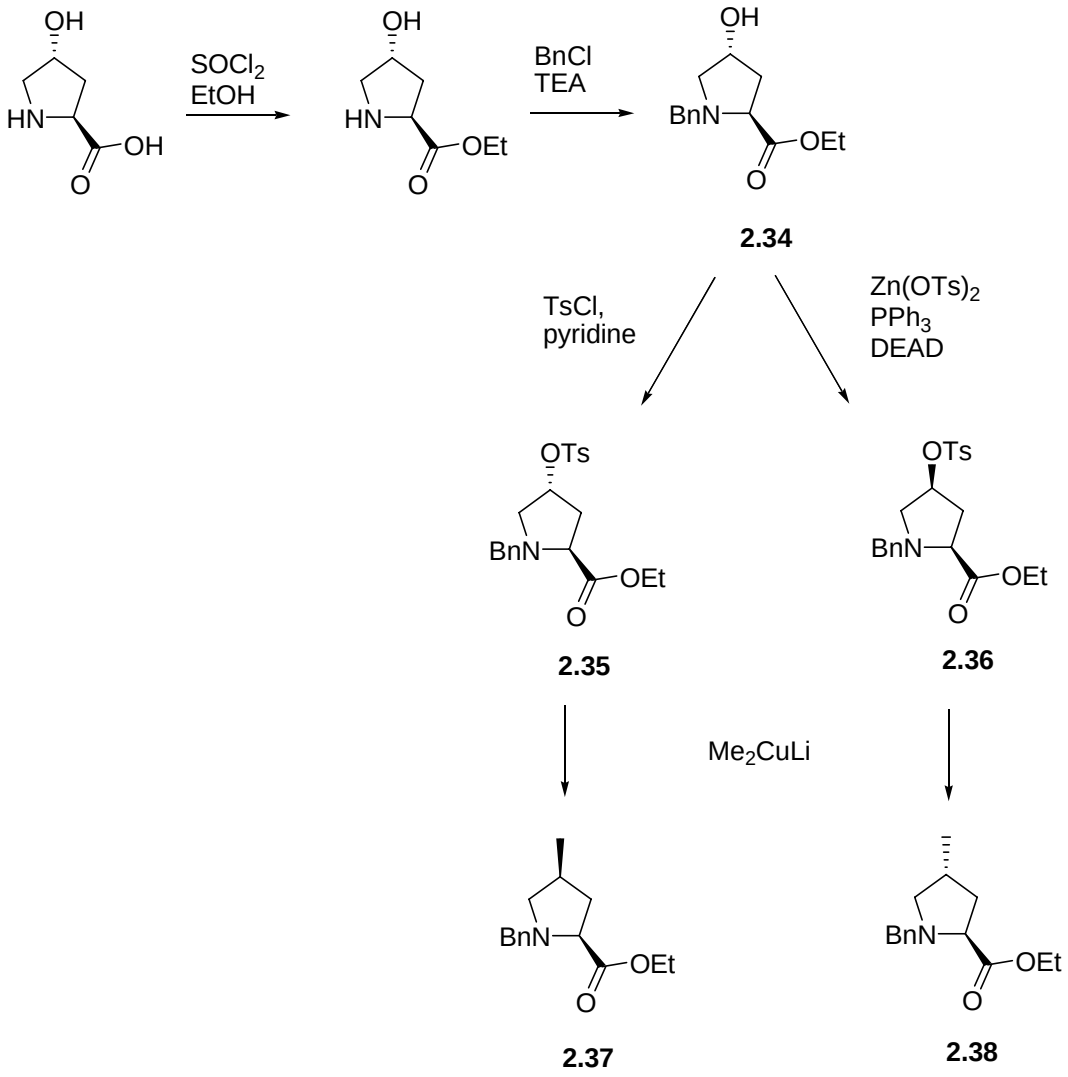
Scheme 2.22 – Del Valle's stereoselective route to 4-methylproline

This method is also very powerful, allowing late stage stereochemical divergence from a common precursor. It does suffer some drawbacks however. Whilst the stereoselectivity observed with Crabtree's directed hydrogenation was very high (> 40:1 of the *trans*-isomer to *cis*-), the facial selectivity observed with heterogeneous catalytic hydrogenation was significantly lower (3:1 of the *cis*- isomer to *trans*-) than that reported by Moody and Krapcho (stereospecific). This is most likely as a result of lower steric hindrance being exerted by the alcohol compared to carboxyl derivatives. In addition, this route is relatively lengthy, requiring redox manipulation and functional group protection.

Of the routes reviewed above, the last two[85,86] were the most appropriate for the purpose of obtaining reference samples of all four diastereoisomers of 4-methylproline. The route of Heindl was chosen initially, as it offered a relatively short synthesis and involved readily available and inexpensive reagents (**Section 2.3.2**). In the present work, this route proved challenging, with low and irreproducible results obtained at the crucial γ methylation reaction. As a result, a modified version of the route of Del Valle was successfully utilised to obtain the four diastereoisomers of 4-methylproline (**Section 2.3.3**).

2.3.2 Attempted synthesis of 4-methylproline via Heindl's method

The synthesis of 4-methylproline by the route of Heindl *et al*. begins with protection of both the acid and the amine functional groups of *trans*-4-hydroxy-proline. The route was initially undertaken with *trans*-4-hydroxy-L-proline to establish the route as this is commercially available and the most inexpensive stereoisomer. The first step was to protect the carboxylic acid moiety as an ethyl ester (**Scheme 2.23**). This was readily achieved by addition of thionyl chloride to an ethanolic suspension of the amino acid.[89] Subsequent *N*-benzylation by a nucleophilic substitution reaction of the amine on benzyl chloride gave the protected amino acid **2.34**. Good yields were obtained at each protection step and ^{1}H NMR data of **2.34** were in excellent agreement with those reported by Heindl *et al*.



Scheme 2.23 – Variation on Heindl and co-workers synthesis of protected 4-methylproline

It is at this point that the stereochemical divergence in the route begins. The eventual stereochemistry of the γ-methyl group on 4-methylproline is determined by the stereochemistry of the activated alcohol generated from **2.34**, as the alkylation of the activated alcohol should proceed reliably with inversion. Therefore this method relies on two divergent methods to generate the *trans*- and *cis*- diastereoisomers, **2.35** and **2.36**. A simple direct tosylation gives access to the tosyl derivative **2.35**. This reaction proceeded in a similarly moderate yield (57%) to that reported by Heindl *et al*. (67%) with tosyl chloride. In an attempt to improve the yield, the reaction was repeated with the

alternative tosylating reagent tosic anhydride, however, this did not offer any improvement on the yield (48%). The ^{1}H NMR data of the product were in good agreement with those reported and the molecular formula was confirmed by high resolution mass spectrometry.

For generation of the diastereoisomer of **2.36**, Heindl *et al*. relied on a three step conversion of **2.35**. This involved first the displacement of the tosylate group with the tetrabutylammonium salt of acetate which proceeds with inversion. The acetate group generated is hydrolysed under basic conditions to generate the protected *cis*-4-hydroxyproline and the alcohol is tosylated as before to give protected *O*-tosyl-*cis*-4-hydroxyproline, **2.36**. Whilst this four-step route from alcohol **2.34** to inverted tosylate **2.36** is effective and relatively high yielding, it adds several steps to the overall synthesis and is therefore not very efficient.

A one-step procedure to introduce tosylate groups with inversion of stereochemistry relative to the starting alcohol was first described by Galynker and Still,[90] and has subsequently been used extensively for bond formation with net retention of stereochemistry (literature examples given by Anderson *et al*.).[91] This procedure is a variant of the Mitusnobu reaction, in which a nucleophile displaces an alcohol which is activated as an alkyloxyphosphonium salt (see **Section 2.2.4** for further discussion of the mechanism). Galynker and Still demonstrated that the tosylate anion could be used as a nucleophile under Mitsunobu conditions, although the counter-ion used proved to be of importance, with zinc tosylate being the reagent of choice. The reaction is high yielding and reliably occurs with inversion of stereochemistry.

The use of this procedure would allow the four-step tosylation route used by Heindl and co-workers to be abbreviated to a single step, clearly offering a significant improvement on the original synthesis. The Mitsunobu tosylation procedure was therefore attempted on alcohol **2.34**. Pleasingly, the reaction proceeded smoothly to afford the tosylation product, **2.36**, with inversion of stereochemistry (as judged by comparison of ^{1}H NMR data – which were in excellent agreement with the literature data for the *cis*-

diastereoisomer **2.36**) in a moderate 52% yield. Whilst the yield was not high, it is comparable with that observed during the standard (retentive) tosylation (to give **2.35**), is similar to the combined yield over the reported four-step route (49%) and offers the clear advantage of a reduced number of synthetic manipulations.

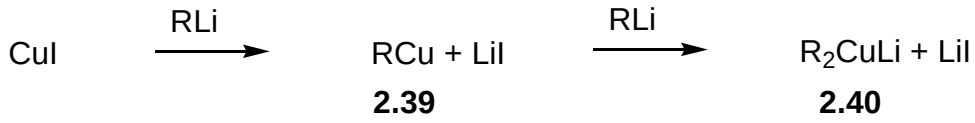
With both diastereoisomers of the activated hydroxyproline derivative in hand (**2.35** and **2.36**) the methylation reaction could be undertaken. With two potential electrophilic sites available on these proline derivatives, an ester and an activated alcohol, careful choice of the nucleophile is necessary. For example a Grignard reagent would be inappropriate as such a reactive nucleophile would be expected to react very readily at the carbonyl of the ester function at the same time or even in preference to the tosylate sp^3 carbon. Heindl's method takes advantage of a mild methyl nucleophile, the Gilman reagent, to improve regioselectivity.

The Gilman reagent is an example of an organocuprate reagent; reagents which have found a great deal of utility in a range of different synthetic manipulations due to their differing reactivity patterns to traditional organometallics such as organolithium or Grignard reagents.[92] They are considered to be soft nucleophiles, with the copper-carbon bond displaying relatively low ionic character and are considerably less basic than their organoalkali counterparts. As a result they display a more controlled reactivity with fewer competing side-reactions such as elimination reactions. They are formed from the corresponding organoalkali compound by reaction with a copper(I) salt.

The first example of an organocopper reagent was described by Kharasch and Tawney. They investigated the reaction of a Grignard with a conjugated ketone and observed a change in reaction outcome on addition of catalytic quantities of various metal salts, in particular copper.[93] They found that the usual tendency to form 1,2-addition products observed with Grignard reagents became a Michael-type 1,4-addition on inclusion of catalytic copper. The 1,4-alkylation of unconjugated ketones has become one of the principal synthetic applications of organocuprates.[94] The other major application of organocuprates is in substitution reactions of halides and activated alcohols where they

behave as efficient nucleophiles and display fewer competing side-reactions. The application of organocuprate 1,2-addition on carbonyls is rare,[94] although it is known. In particular it has been used in asymmetric addition to chiral aldehydes, where copper's ability to coordinate multiple ligands allows chelation controlled addition and steric bulk allows non-chelation controlled addition.[95]

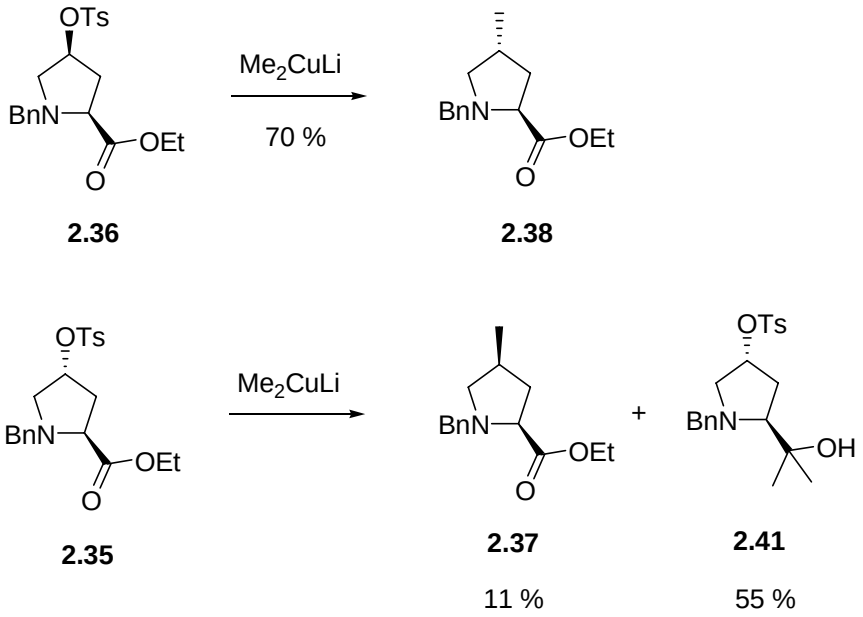
Whilst the first reports of organocopper reagents used catalytic quantities of copper salt, the use of stoichiometric quantities has been found to give more reliable results.[92] There are a number of different forms of organocopper reagents which differ in the ratio of organoalkali to copper(I). The addition of one equivalent of methyl lithium to copper iodide forms an insoluble monoorganocopper reagent of type **2.39** (**Scheme 2.24**) which generally has no synthetic utility. The addition of a second equivalent of methyl lithium, however, leads to the organic soluble organocuprate of type **2.40**, which is highly reactive and is the most commonly used form of organocopper reagent.[96]



2.39 **2.40**

Scheme 2.24 – Generation of organocopper reagents

The above brief review of organocuprate chemistry demonstrates their utility in substitution reactions and benefits they offer over organoalkali reagents. In addition, their limited reactivity in 1,2-addition to carbonyl groups explains Heindl and co-worker's choice of the Gilman reagent for the substitution of the activated alcohol in their synthesis of 4-methylproline derivatives.[85]



Scheme 2.25 – Methylation results reported by Heindl *et al.*

They reported a respectable yield of 70% during methylation of the *cis*- tosyl **2.36** to generate the protected *trans*-4-methylproline **2.38** (**Scheme 2.25**). However, the methylation of the protected *trans*- tosyl **2.35** to generate the *cis*- diastereoisomer **2.37** proceeded in only 11% yield. The major product isolated from this reaction was reported as the alcohol **2.41**, the product of a 1,2-carbonyl double addition. This latter result is best explained by steric hindrance due to the ester functional group slowing attack at the activated alcohol carbon sufficiently to allow the slower 1,2-carbonyl addition reaction to compete effectively for the Gilman reagent. It is therefore evident that a 1,2-carbonyl addition to an ester is also possible in this system.

The methylations of **2.35** and **2.36** were carried out under the same conditions used by Heindl and co-workers. Since organocuprates are relatively unstable, the reactions are carried out at low temperatures, with the cuprate initially formed at -20ºC and then the reaction performed at 0ºC. The instability of the organocuprates also requires a large excess to be used, and Heindl reported that the use of 7 equivalents gave the best results. The reaction products were analysed by LCMS or NMR spectroscopy. In the case of methylation of **2.35**, LCMS of the crude reaction product showed mainly the product of carbonyl dimethylation, **2.41**, with no desired substitution product observed. In the

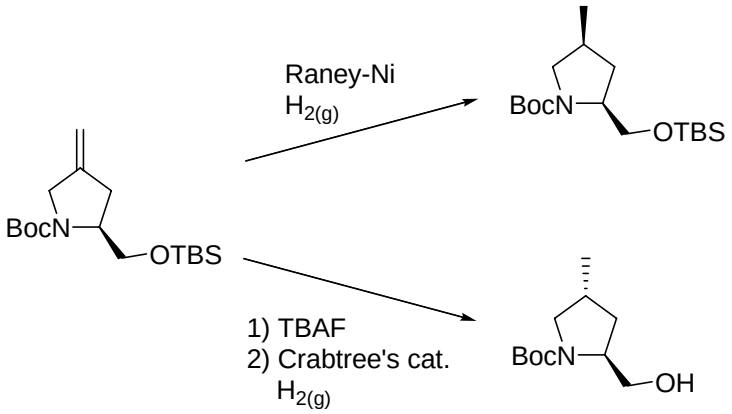
reaction of **2.36** to give the *trans*- diastereoisomer, **2.38**, the ^{1}H NMR spectrum of the crude material showed that no starting material remained and purification of the crude residue gave the desired product in 8% yield. These were disappointing results, since one diastereoisomer (the *cis*-) was not formed at all and the other was formed in only 8% compared to the literature report of 70%.

In order to improve these results, some adjustments were made to the reaction conditions. These were largely attempted using **2.35** to make the *cis*- diastereoisomer, as this had not been formed at all under the literature conditions. The main parameter examined was temperature. Organocuprates are known to be active at temperatures as low as –78ºC,[97] so lower temperatures were explored in the hope that the 1,2-carbonyl dimethylation could be slowed sufficiently to allow the desired substitution to compete. The reaction was carried out as Heindl reported except the temperature was varied between –40 and 0ºC (the temperature during organocuprate pre-formation was the same as that employed for the reaction) and the reaction times were extended to around 6 hours. It was found that at temperatures below -12ºC only starting material was recovered. At 0ºC the desired product **2.37** was isolated in 5% yield on one occasion, however this result proved not to be reproducible, with carbonyl addition the only reaction on most occasions. The methylation of **2.36** was also repeated at different temperatures, and gave a respectable yield of 75% of **2.38** on one occasion (-8ºC) but this result could not be repeated.

The irreproducibility of the methylation reactions in our hands, and particularly the low yield achieved when forming the *cis*- diastereoisomer detracts significantly from the utility of this route to 4-methylproline. Whilst further efforts could have been made to optimise reaction conditions, it was decided to attempt the route of Del Valle as an alternative route.

2.3.3 Synthesis of 4-methylproline based on Del Valle's method

The method of Del Valle and Goodman provides access to all four diastereoisomers of 4-methylproline via a stereoselective route, with reasonably late-stage stereochemical divergence.[86] This divergence relies on two different stereoselective, catalytic hydrogenations of an exomethylene double bond to create the γ methyl group (**Scheme 2.26**). The exomethylene group is introduced via modification of 4-hydroxyproline. A number of redox and protection reactions are utilised in the route, whose roles are explained later.



Scheme 2.26 – Stereochemical divergence in the synthesis of 4-methylproline by Del Valle and Goodman

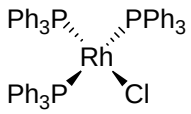
Traditional heterogeneous catalytic hydrogenation using Raney nickel was employed to obtain the *cis*- diastereisomer with a modest stereoselectivity of 3:1. This stereoselectivity is a result of differential steric environments on either face of the molecule as a result of a -CH_2OTBS group whose stereochemistry is defined by that of the optically pure starting 4-hydroxyproline. The sterically encumbered face will not be able to approach the surface of the heterogeneous catalyst as easily and will therefore not be hydrogenated as easily. This modest selectivity was the result of optimisation, with three heterogeneous catalysts examined (Pd/C, Rh/C and Raney nickel). As mentioned earlier, the degree of stereoselectivity observed is not as great as the stereospecific catalytic (Pd/C) hydrogenation of an exomethylene derivative of pyroglutamic acid (**Scheme 2.17**) reported by Moody *et al*. This may be partly due to the greater ring

tension induced by the sp^2 lactam locking the five-membered ring into such a conformation that hydrogenation from the same side as the α ester group is impossible. It is also likely that the ester offers greater steric bulk than a $-CH_2OTBS$ functional group, which, whilst bulky, may be able to adopt a position which does not effectively hinder the approach of the catalyst to this face.

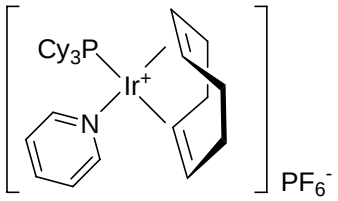
Crabtree's homogeneous hydrogenation catalyst is employed to obtain the *trans*-diastereoisomer by a directed hydrogenation. Directed homogeneous hydrogenation is arguably one of the most important tools in the arsenal of asymmetric synthesis.[98] Its importance was acknowledged by the award of the 2001 Nobel Prize to Knowles and Noyori for the development of chiral homogeneous catalysts (rhodium or ruthenium metal complexes) capable of directing hydrogenation selectively to one face of a pro-chiral alkene. These catalysts require the presence of a functional group near to the alkene which is also capable of coordinating to the metal. The chiral ligand spatially arranges the alkene and hydrogen selectively around the metal, so that hydrogenation occurs on the desired face of the alkene when the appropriate ligand is chosen. Surprisingly, mechanistic studies showed that it is actually the less stable diastereoisomeric metal-substrate complex which defines the eventual product.[99] This occurs because the less stable complex reacts with a molecule of hydrogen more readily than the more stable complex, and as hydrogen addition is the rate-limiting-step, this step determines the eventual reaction pathway.

When hydrogenating chiral alkenes with a coordinating functional group, a chiral catalyst may no longer be required as advantage can be taken of the stereochemistry inherent in the substrate (substrate directed hydrogenation). Since both the alkene and the additional coordinating group (*vide infra*) are bound to the metal centre, *cis*- delivery of hydrogen is enforced. Evidently only one diastereoisomer is accessible by this approach. This concept was first proved by Thompson and McPherson using Wilkinson's homogeneous rhodium catalyst, **2.42**.[100] However, as commented by the authors, the potential of this approach was limited by the low activity of this catalyst since those instances where it might be applied usually involve alkenes whose degree of substitution is significant.

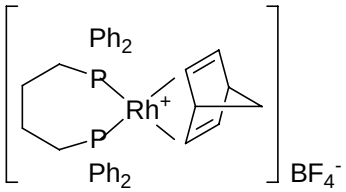
Therefore the popularisation of this approach had to wait until the advent of more active homogeneous hydrogenation catalysts such as the iridium catalyst [Ir(cod)pyr(PCy_3)]PF_6, **2.43**, known as Crabtree's catalyst. This catalyst was developed by Crabtree, Felkin and Morris,[101] with initial interest stemming from the observation that it was highly active; capable of catalysing the hydrogenation of very hindered, tri- and tetra- substituted alkenes not hydrogenated by other catalysts.[87] The catalyst's application in directed hydrogenation was subsequently discovered and independently reported by Crabtree and Davis,[102] and Stork and Kahne.[88] The rhodium complex **2.44** has also been demonstrated to be capable of catalysing directed hydrogenation, however it displays significantly lower activity. The stability and ease of use of Crabtree's catalyst, its high activity, commercial availability and the reliably high enantiomeric excesses observed with it have led to its popularity in directed homogeneous hydrogenations of chiral alkenes.



2.42

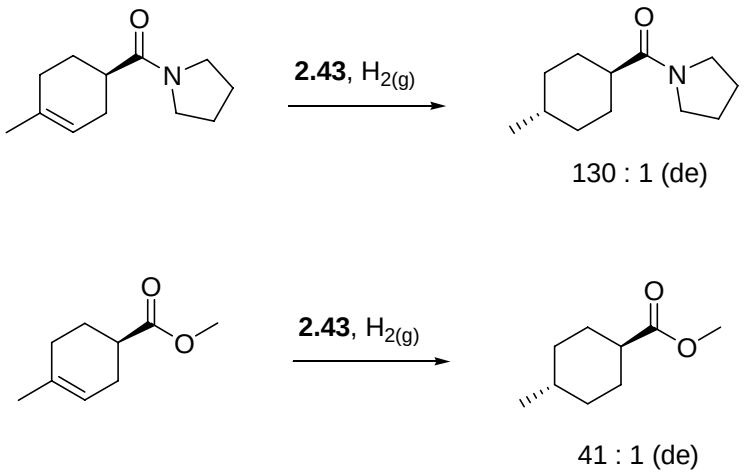


2.43



2.44

Many functional groups are known to be capable of directing hydrogenation with Crabtree's catalyst. The initial studies employed alkenes with a hydroxyl group in close proximity, and the hydrogenations generally proceeded with excellent diastereoselectivity.[88,102] However, other polar functionalities capable of donating a lone pair to a metal are also able to direct hydrogenation with Crabtree's catalyst. Proximal esters and amides in particular are known to be capable of inducing high stereoselectivity.[103,104] It was demonstrated by Schultz and McCloskey that amides are the better directing group, with higher diastereoisomeric excess observed (**Scheme 2.27**), however esters still display a good degree of stereoselectivity.

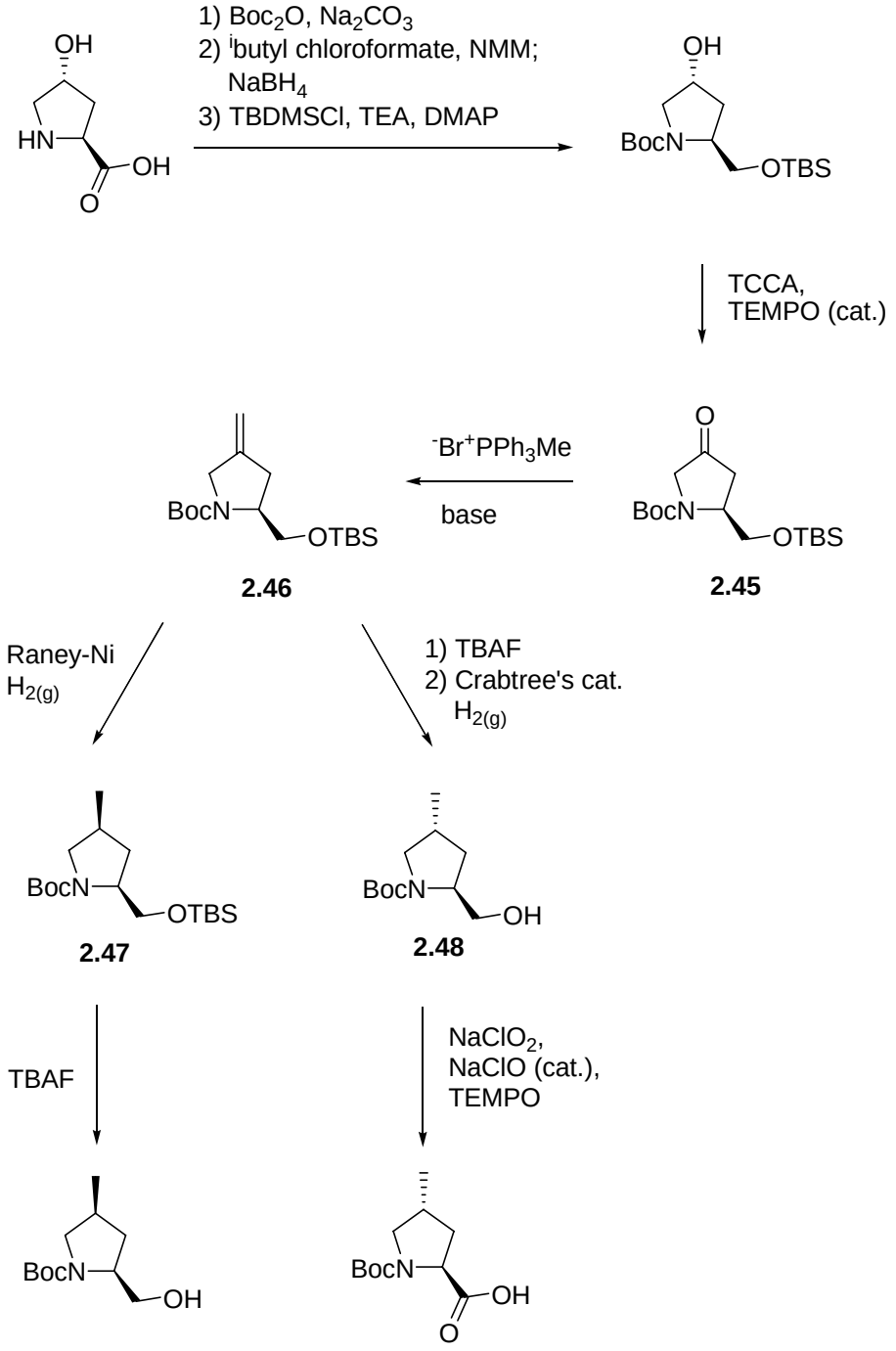


Scheme 2.27 – Comparison of amide and ester directing groups in Crabtree's catalysed hydrogenation[103]

Del Valle and Goodman report high diastereoisomeric excess when using Crabtree's catalyst (> 40:1) to form derivatives of *trans*-4-methylproline. In their work, the principal aim was to generate 4-substituted prolinols for use in peptidomimetic research programs, rather than the 4-substituted proline required in this present work. As a result, the synthetic route chosen by the authors involved reduction of the carboxylic acid moiety of protected 4-hydroxyproline to generate an alcohol, which is the classic directing group used in directing asymmetric hydrogenation. Details of the synthetic steps in Del Valle and Goodman's route are shown in **Scheme 2.28**.

After protection of the amine of *trans*-4-hydroxyproline with a Boc group, the carboxylic acid is reduced to an alcohol in a two-step process via the intermediacy of a mixed anhydride. The authors found that the reduction of the anhydride was easier when working on a large-scale, proceeding with less solvent and offered a less complicated workup than the more traditional reduction of an ester route. The requirement for oxidation of the γ hydroxyl group required protection of this newly formed alcohol, and was therefore protected as a silyl ether under standard conditions. Oxidation of the γ alcohol was carried out using trichloroisocyanuric acid as the stoichiometric oxidant in the presence of catalytic quantities of TEMPO to give ketone **2.45**.[105] Olefination of the

ketone was then achieved using classic Wittig conditions. This sequence provided the crucial exomethylene precursor, **2.46**, for the divergent hydrogenations.



Scheme 2.28 – Details of the synthetic route to 4-methylproline by Del Valle and Goodman

Exomethylene **2.46** was hydrogenated under heterogeneous conditions to generate the protected *cis*-4-methylprolinol, **2.47**. As discussed earlier, the use of Raney nickel gave the best stereoselectivity of the heterogeneous catalysts examined, giving a 3:1 mixture of *cis*- and *trans*- diastereoisomers. The hydrogenation was carried out before cleavage of the silyl ether presumably due to the increased steric bulk of this group compared to an unprotected alcohol, thus optimising facial selectivity in the delivery of hydrogen at the catalyst surface. Cleavage of the silyl ether was then carried out after the hydrogenation using standard, basic fluorinating conditions (TBAF).

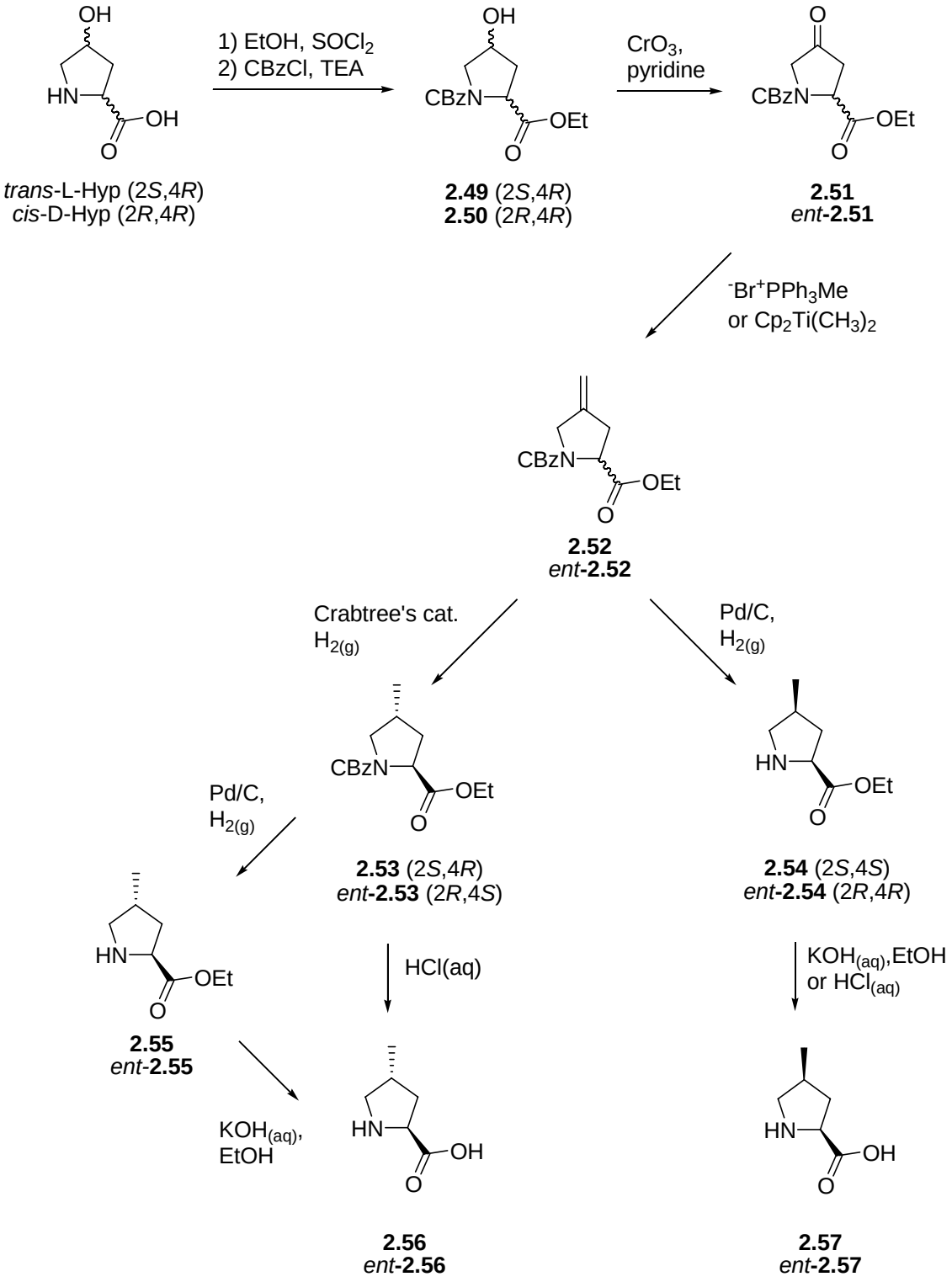
The Crabtree's hydrogenation to give the *trans*- diastereoisomer must be carried out after removal of the silyl ether protecting group, since the alcohol must be able to coordinate to the iridium catalyst to direct the hydrogenation. The hydrogenation of the alcohol then proceeded in good yield to give **2.48** and with excellent stereoselectivity of >40:1 *trans*- to *cis*- diastereoisomer.

Whilst the authors had successfully obtained the 4-substituted prolinols, which was their aim, they also undertook the oxidation of one of the alcohols, **2.48**, to obtain the *trans*-4-substituted proline amino acid. They carried out the oxidation using a buffered solution of sodium chlorite and sodium hypochlorite with catalytic TEMPO. The authors did not cleave the amine protecting Boc group, but this has been readily achieved in related compounds using standard acid treatment.[78]

As discussed earlier, this route is a powerful method to generate 4-methylprolines with moderate to excellent stereoselectivities. It does suffer some drawbacks, in that it is a relatively long route, involving a number of changes of oxidation state and protection steps. In addition, the stereoselectivity observed for the *cis*- series is not as high as might be hoped. Consideration was therefore given to amending one crucial aspect of the synthesis in the hope that it would improve both of these problems. Del Valle and Goodman's use of an alcohol as the directing group in the Crabtree's hydrogenation requires the inclusion of a reductive and subsequently a reoxidative step in the synthetic route. Whilst very successful in directing the *trans*- hydrogenation, it is likely that the

more flexible -CH_2OTBS functional group results in the diminished stereoselectivity during the formation of the *cis*- diastereoisomer. If an ester functional group were employed as the directing group, it was speculated that the stereoselectivity observed in the heterogeneous hydrogenation might be improved to a level similar to that observed by Moody *et al*. (*vide supra*, **Scheme 2.17**).[78] In addition, this would remove the need to reduce and reoxidise the carboxylic acid moiety, and would thus abbreviate the synthesis. It has been shown that esters can be excellent directing groups in asymmetric hydrogenations,[104] although their ability to direct is slightly less than equivalent alcohols. Nonetheless, it would not be anticipated that there would be a significant change in the level of stereoselectivity during the formation of the *trans*- series. With this change in mind, the amended synthetic strategy shown in **Scheme 2.29** (only one enantiomeric series, L-, is shown) was developed.

In this amended strategy, after esterification and amine protection, oxidation would be carried out to give the ketone. PCC (generated *in situ*) was chosen as oxidant since it was readily available in-house, although it has many drawbacks, such as high stoichiometry requirements and environmental toxicity. As in Del Valle and Goodman's synthesis, a Wittig or Petasis (*vide infra*) reaction would be used to install the exomethylene function. Stereodivergent hydrogenations would then be carried out on the exomethylene **2.52**, by the methods employed by Del Valle and Goodman. Finally simultaneous deprotection of both amine and carboxylate groups would be carried out under acidic conditions.



trans-L-Hyp (2*S*,4*R*)
cis-D-Hyp (2*R*,4*R*)

2.49 (2*S*,4*R*)
2.50 (2*R*,4*R*)

2.51
ent-**2.51**

2.52
ent-**2.52**

2.53 (2*S*,4*R*)
ent-**2.53** (2*R*,4*S*)

2.54 (2*S*,4*S*)
ent-**2.54** (2*R*,4*R*)

2.55
ent-**2.55**

2.56
ent-**2.56**

2.57
ent-**2.57**

Scheme 2.29 - Alternative route to 4-methylprolines

This route was carried out using both *trans*-4-hydroxy-L-proline and *cis*-4-hydroxy-D-proline; the least expensive of the four diastereoisomers of 4-hydroxyproline. Since the stereochemistry of the γ position in these starting materials is lost during oxidation to form a ketone, the initial stereochemistry is unimportant. After esterification (as discussed earlier), protection of the amine using CBz chloride under basic conditions afforded the protected 4-hydroxyprolines, **2.49** and **2.50**, in approximately quantitative yields, which were characterised by NMR spectroscopy and high resolution mass spectrometry. The specific optical rotation of the alcohols **2.49** and **2.50** were -55 and +20º respectively (no literature values available).

The oxidation of the alcohols to give ketones **2.51** and *ent*-**2.51** proceeded in good yields (85% and 87% respectively) using 13 equivalents of PCC (generated *in situ*) (characterised by NMR spectroscopy and high resolution mass spectrometry). The measured specific optical rotations of **2.51** and *ent*-**2.51** were of equal value and opposite sign (-4 and +4º respectively, no literature values available). In an attempt to use a more environmentally benign oxidative conditions, oxidation of **2.49** using IBX in DMSO was examined. The oxidation proceeded, but gave only a 36% yield.

The olefination of **2.51** and *ent*-**2.51** was undertaken using the Wittig reaction under the same conditions as those described by Chirgadze.[79] This involved formation of the methylenetriphenylphosphorane ylide from the methylenephosphonium iodide (itself made by reaction of triphenylphosphine with methyl iodide) using potassium ^{t}butoxide as base. The product was obtained in a disappointing 26% yield for **2.52** and 30% for *ent*-**2.52**. This result was also found to be quite variable, frequently giving lower yields. The product was characterised by NMR spectroscopy and high resolution mass spectrometry. No NMR data were given for **2.52** by Chirgadze *et al*., precluding direct comparison, however 2D NMR experiments confirmed the assignment of the product. The optical rotations observed for the L and D forms (**2.52** and *ent*-**2.52,** observed at 589 nm) were very small, to the extent that no accurate value could be assigned. This result boded ill for the optical purity of the product, since the Wittig reagent is mildly basic and could therefore theoretically epimerise the α carbon of the proline derivative. This seems

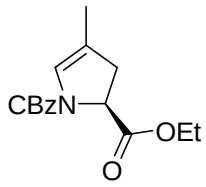
unlikely, however, given the common use of the Wittig reaction on amino acids with no observed α epimerisation. In the absence of literature values (none given by Chirgadze *et al*.) for direct comparison, the optical purity of the product was assumed to have been conserved during the olefination.

Stereodivergent hydrogenations were undertaken on exomethylenes **2.52** and *ent*-**2.52** to form **2.53**, **2.54** and their enantiomers. Heterogeneous catalytic hydrogenation was carried out on **2.52** and *ent*-**2.52** using palladium on carbon as the catalyst at atmospheric pressure. This proceeded cleanly to give protected *cis*-4-methylprolines **2.54** and *ent*-**2.54** in good yields of 80% and 71% respectively. After passing the crude reaction mixture through a pad of Celite, a ^{1}H NMR spectrum of the product showed trace impurities were present, however no further purification was attempted at this stage. 2D NMR data and high resolution mass spectrometry confirmed the identity of the product. Due to limitations of scale, no optical rotation data was obtained at this stage. The presence of small amounts of a related compound were in evidence from the ^{1}H NMR spectrum, which was assumed to be the diastereoisomeric side-product and is discussed further later.

The homogeneous Crabtree's catalysed hydrogenation of exomethylenes **2.52** and *ent*-**2.52** were carried out under standard conditions (DCM as solvent and atmospheric pressure). This gave the protected *trans*-4-methylprolines **2.53** and *ent*-**2.53** in good yields (84% and 80% respectively) after several days. The structure of the product was confirmed by NMR data (discussed further later) and high resolution mass spectral data. Both the ^{1}H and ^{13}C NMR spectra displayed isomerisation around the carbamate, with doubling of peaks observed. Only trace impurities were observed in the ^{1}H NMR spectrum, which were tentatively assigned as the product diastereoisomer, suggesting that the reaction had proceeded with good stereoselectivity. Again, due to limitations of scale, no optical rotation data were obtained at this stage.

Close monitoring of the hydrogenation reaction showed that after one day, no starting exomethylene **2.52** remained, but only small amounts of product **2.53** had been formed.

The major compound present was the product of a double bond migration from exomethylene to an endocyclic position, **2.58** (also observed in *ent*- series). High resolution mass spectrometry showed the mass of the pseudomolecular ion of **2.58** was isobaric with the starting material, **2.52**, suggestive of an isomerisation product. ^{1}H NMR data (**Figure 2.6**, 1:1 mixture of isomers around the carbamate observed) established the presence of broadened singlet olefinic protons (δ 6.40 and 6.32) and broadened singlet methyl groups (δ 1.70 and 1.68). COSY NMR spectroscopy supported the proposed structure, with correlations between the H-2 (δ 4.70 and 4.64) and H-3 (δ 2.99 and 2.53) protons, as well as an allylic coupling between the H-5 proton and H-6 protons.



2.58
ent-**2.58**

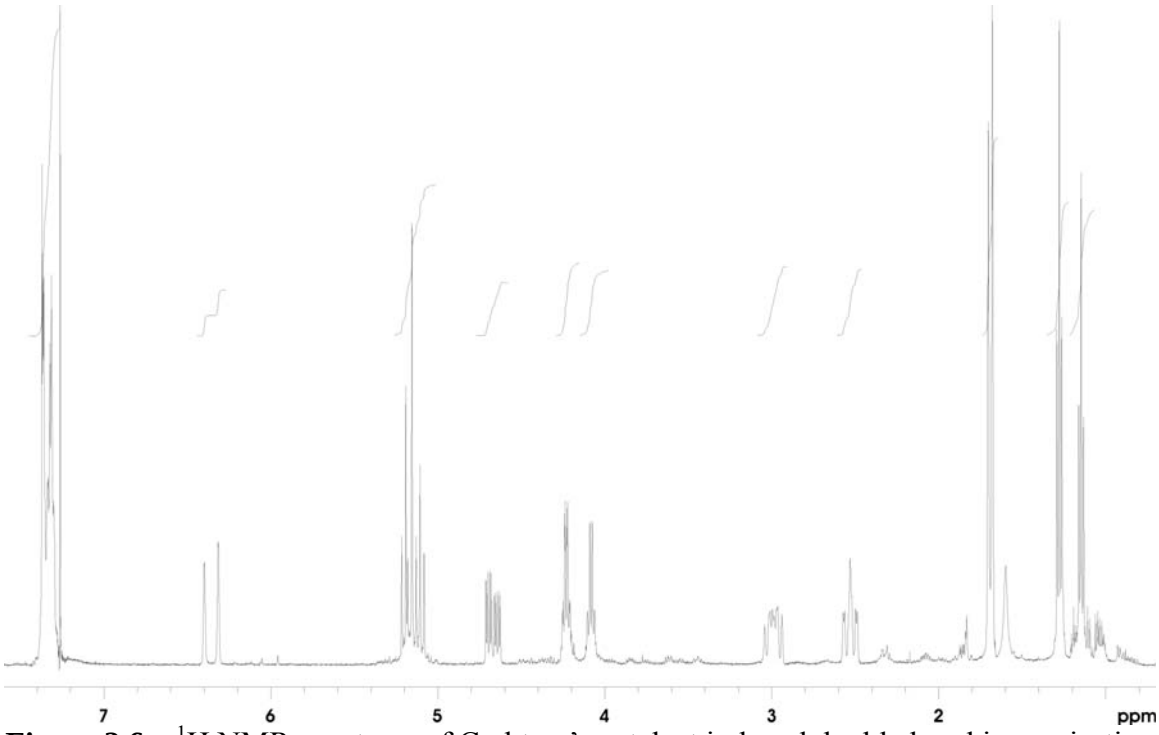
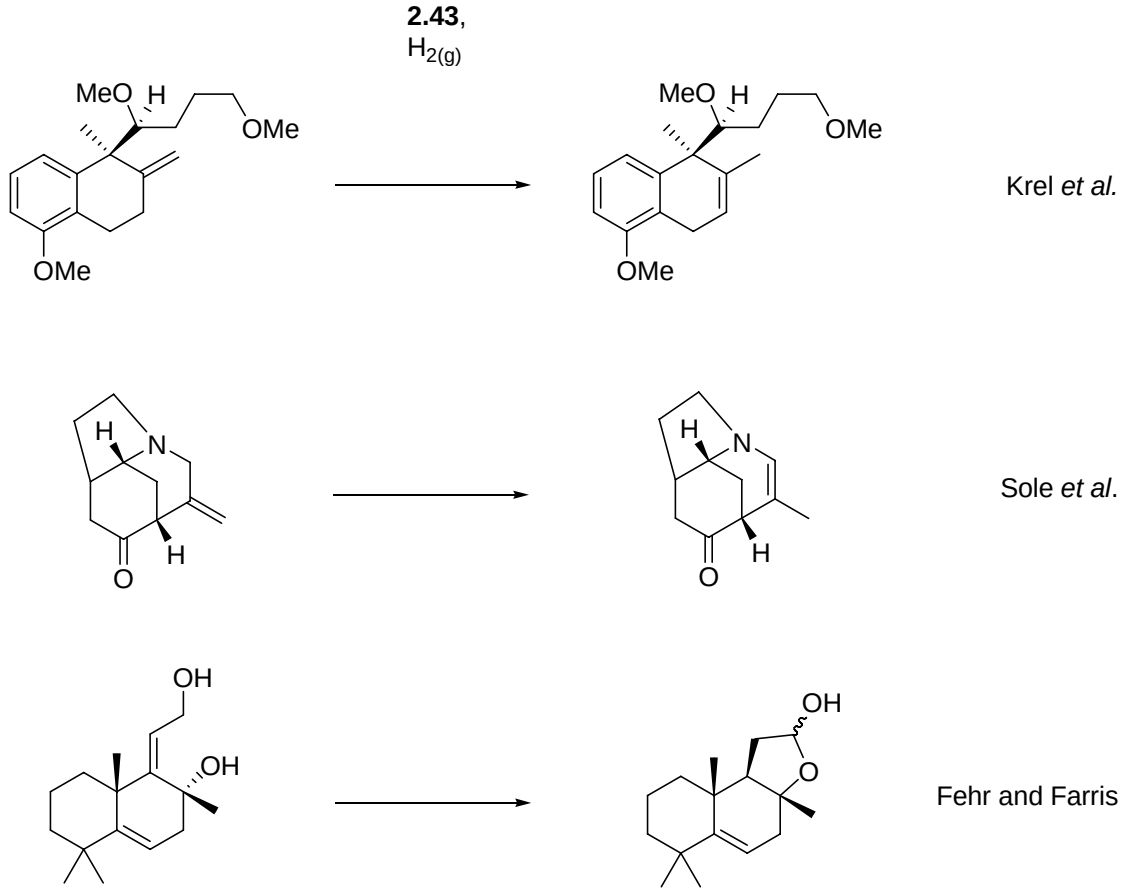


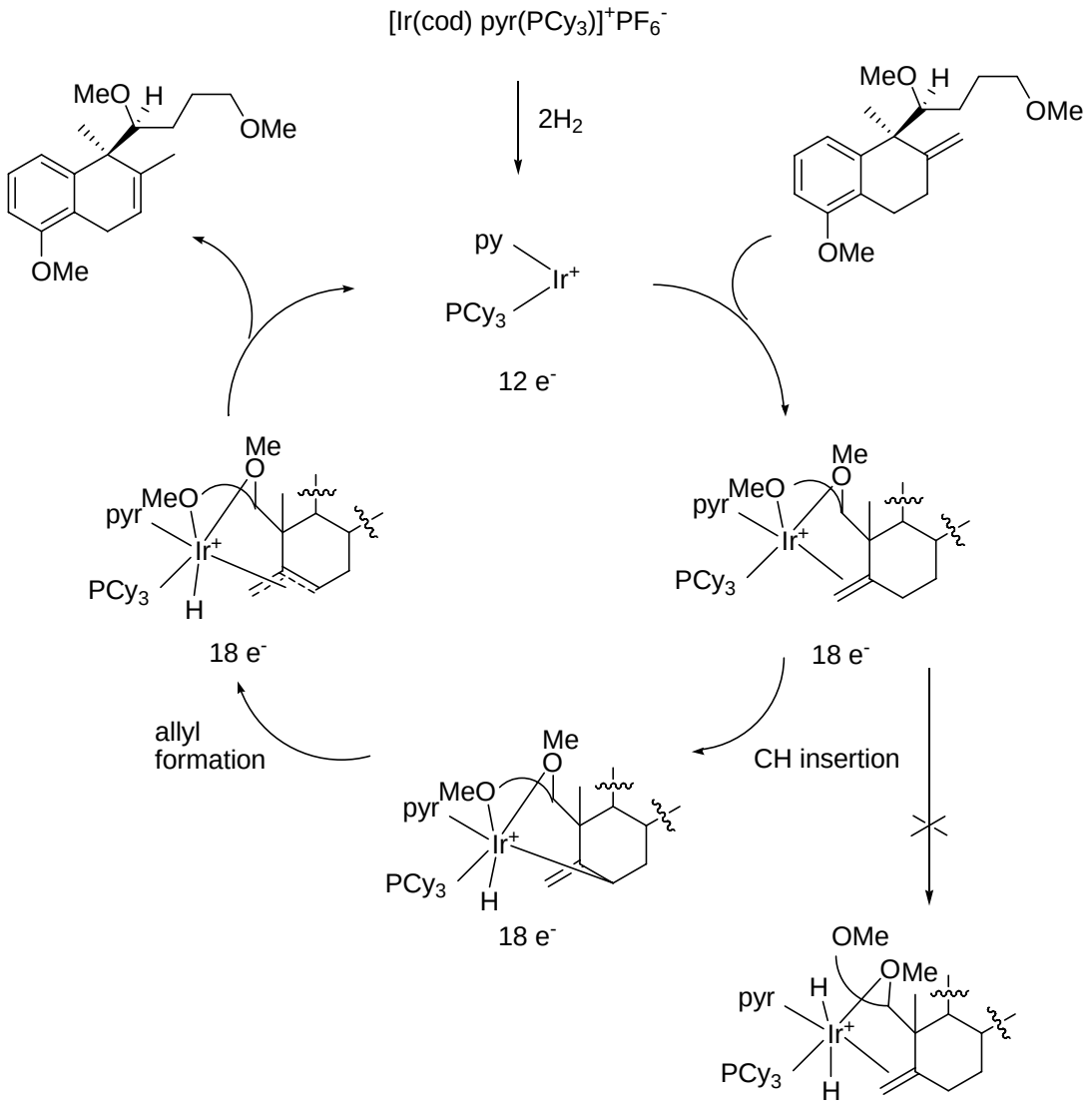
Figure 2.6 – ^{1}H NMR spectrum of Crabtree's catalyst induced double bond isomerisation product

Double bond isomerisation has been observed under Crabtree's hydrogenation conditions, first by Krel *et al*. and subsequently by Solé and co-workers and by Fehr and Farris (**Scheme 2.30**).[106,107,108]



Scheme 2.30 – Crabtree's catalyst (**2.43**) induced double bond isomerisation

In the first two examples, an exocyclic methylene migrates to the more stable endocyclic isomers. In the final example the exomethylene migrates onto an alkyl side chain, generating an aldehyde, which is subsequently attacked intramolecularly by a tertiary alcohol to yield a lactol. The first group to describe the phenomenon, Krel *et al*., postulated a mechanism for this isomerisation after brief mechanistic studies, as shown in **Scheme 2.31**.



Scheme 2.31 – Krel's proposed mechanism for Crabtree's catalysed double bond migration

The authors suggest that the bulky substrate or a third substrate-metal bond (via the second methoxyl group) prevents the oxidative addition of hydrogen to the metal centre and therefore prevents the hydrogenation pathway. CH insertion and η3 allyl formation can then occur, and on reductive elimination of the substrate, the more thermodynamically stable double bond is formed. Given that hydrogen oxidative addition to asymmetric metal hydrogenation catalysts (*vide supra*) is known to be the rate-determining step, this mechanism seems very plausible. A longer-lived substrate-metal complex has time to undergo rearrangements to more stable isomers, and this

mechanism seems appropriate, both for the other literature examples and for the isomerisation of exomethylene **2.52**.

It is interesting to note that in all the literature cases discussed above, the isomerisation product was stable to the hydrogenation conditions, with no hydrogenated products of the newly formed double bond obtained. In this present work, if the hydrogenation reaction was left for extended periods (or when the isolated endocyclic olefin **2.58** was resubmitted to Crabtree's hydrogenation conditions), hydrogenation to give the protected *trans*-4-methylproline product, **2.53**, eventually went to completion.

Whilst cleavage of the CBz and the ester was later found to be achievable in one step by acid hydrolysis, initially a two step procedure was adopted. A heterogeneous hydrogenation over palladium on carbon was carried out to give the diastereoisomers of **2.55** and *ent*-**2.55**. With all four diastereoisomers of 4-methylproline ethyl ester in hand, hydrolysis of the esters was achieved using basic hydrolysis ($KOH_{(aq)}$ in ethanol). Care was taken to use only a small excess of base, in order to avoid epimerisation at the α carbon.

With the four diastereoisomers of 4-methylproline now available, Marfey's derivatisation was carried out on each. The derivatives were examined by HPLC and the results are shown in **Figure 2.7**.

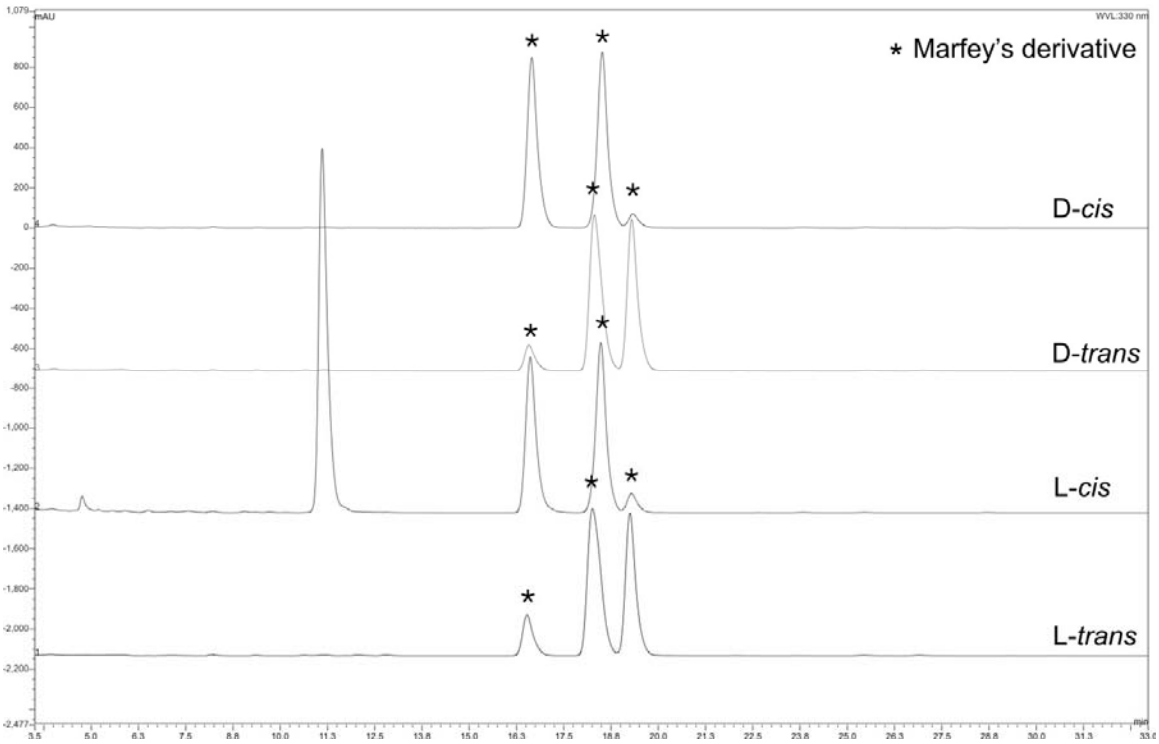
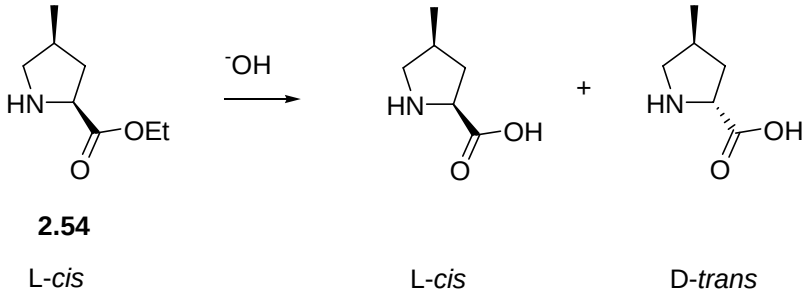


Figure 2.7 – HPLC chromatograms for the Marfey's derivative of the four diastereoisomers of 4-methylproline

Disappointingly, the results of the HPLC analysis of the four derivatives of 4-methylproline reveal that epimerisation of the α carbon had occurred. The two *trans*-diastereoisomers contain two Marfey's peaks of equal area with equal retention times, as do the two *cis*- diastereomers. This result means that at least one step in the synthesis has caused epimerisation. There are two steps during the synthesis that have the possibility of epimerising the α carbon (ie basic conditions); the Wittig reaction and the basic hydrolysis of the ester. The nature of the mixtures of diastereoisomers observed in the products allows the exclusion of the latter. If a base-induced epimerisation of the α carbon had occurred on an optically pure protected 4-methylproline, the diastereoisomeric pairs would be anticipated. For example, if the ester hydrolysis of L-*cis*-4-methylproline ethyl ester, **2.54** caused epimerisation of the α centre, a mixture of L-*cis* and D-*trans*-4-methylproline would be expected (**Scheme 2.32**).



2.54

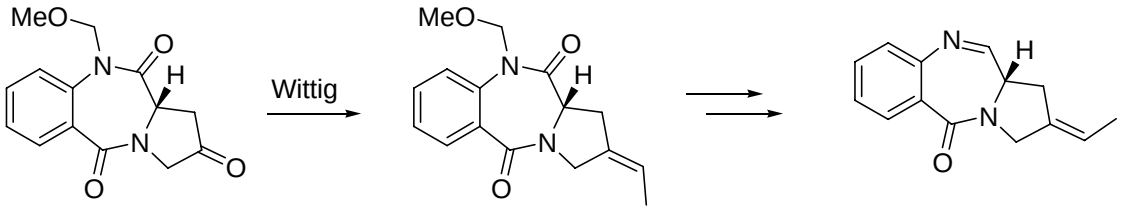
L-*cis* L-*cis* D-*trans*

Scheme 2.32 – Expected products of base induced α-epimerisation of *cis*-4-methylproline ethyl ester

Examination of the HPLC chromatograms of the Marfey's derivatives also revealed that small quantities of diastereomeric pairs were also present, but this will be discussed in more detail later.

Since the observed mixtures of products were enantiomeric pairs (eg L-*trans* and D-*trans*), the epimerisation must have occurred prior to the stereoselective hydrogenations. This makes the Wittig reaction the most likely suspect. Further incriminating evidence was the lack of observable optical activity of the product of the Wittig reaction; exomethylene **2.52**. This step was a reproduction of that described by Chirgadze *et al*., but since the authors gave no optical activity data, it is impossible to say if they also observed racemisation during their reaction. Del Valle and Goodman did not observe any epimerisation of the α carbon during the Wittig reaction of ketone **2.45**. However the divergence of these results from theirs is readily explained by the fact that their ketone precursor was a 1,2-amino alcohol rather than an amino acid, making the α carbon considerably less acidic.

The epimerisation of an amino acid under Wittig conditions had not been anticipated, since this reaction has been commonly used for synthetic manipulation of amino acid containing substrates.[109,110] Indeed, during Mori and co-workers' synthesis of the alkaloid prothracarcin, **2.60**, the Wittig reaction was successfully employed to generate an olefin from a derivative of 4-ketoproline, **2.59**, with a good correspondence between the specific optical rotation of the final synthetic and natural product (**Scheme 2.33**).[109]



2.59 **2.60**

Scheme 2.33 - Wittig reaction utilised during synthesis of Prothracarcin

The Wittig reaction was carried out as per Chirgadze *et al*. ie using solid potassium ^{t}butoxide. The potassium ^{t}butoxide employed was technical grade; however it had been open for an unknown time prior to use. This reagent is highly reactive with water and interaction with atmospheric water may lead to decomposition to give potassium hydroxide and butanol. This opened up the possibility that potassium hydroxide was responsible for the racemisation observed, since it would not be basic enough to deprotonate the phosphonium salt and would therefore remain present after the initial ylide formation step of the Wittig reaction. This explanation may also account for the low yields of reaction observed, since less ylide would be present than anticipated. The Wittig reaction was therefore reattempted employing fresh, commercial potassium ^{t}butoxide THF solution on ketone **2.51**. Again a low yield of 11% was obtained. Hydrogenation over palladium on carbon and hydrolysis of the ester (on this occasion, under acidic conditions), Marfey's derivatisation and HPLC analysis of the product were carried out. The results of this are shown in **Figure 2.8**, with the results of the original Wittig procedure (L-*cis*, **Figure 2.7**) also shown for comparison.

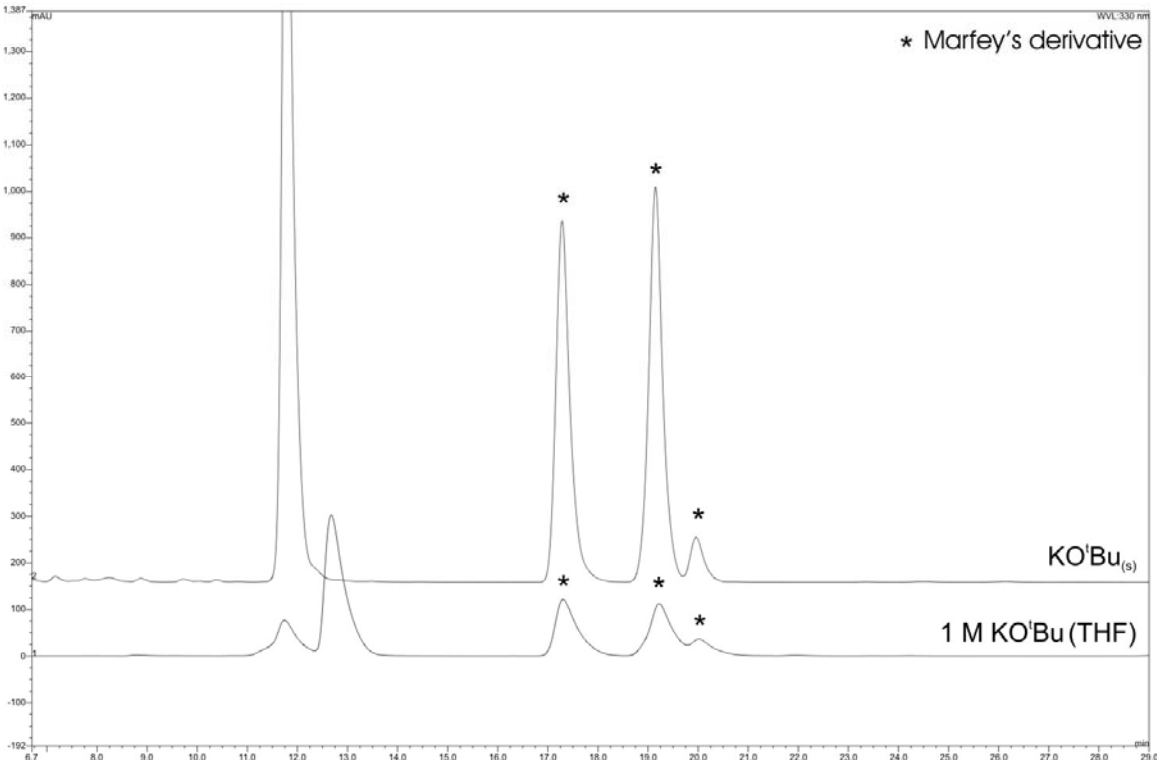


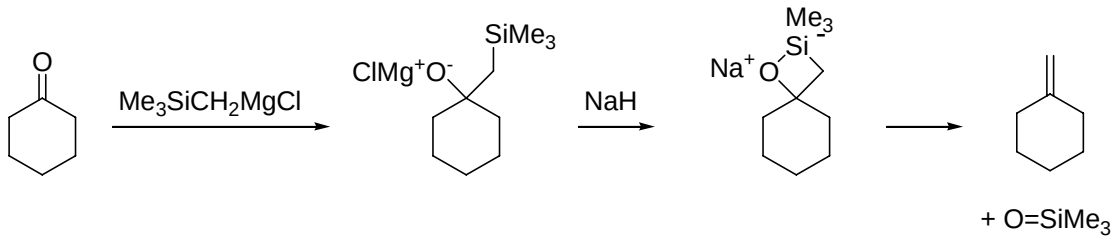
Figure 2.8 – HPLC analysis of Marfey's derivatives of 4-methylproline arising from different Wittig conditions

It can be seen that the same mixture of enantiomers was obtained under both Wittig conditions, whether solid or fresh, commercial solutions of potassium ^{t}butoxide were employed. This indicates that the Wittig reagent itself is sufficiently basic to promote the racemisation of the α carbon of either ketone **2.51** or exomethylene **2.52** or both.

It was clear that a different method of methylenation was required. The olefination of carbonyl groups is most traditionally undertaken employing the classic Wittig reaction. However, a number of other olefination reactions have also been developed in response to the drawbacks inherent in the Wittig reaction.[111] The chief drawbacks are the low yields observed with sterically hindered and cyclic ketones, and the basicity of the ylide which makes it an inappropriate reagent when enolisable ketones are to be olefinated or when other functionalities in the substrate preclude mildly basic conditions.

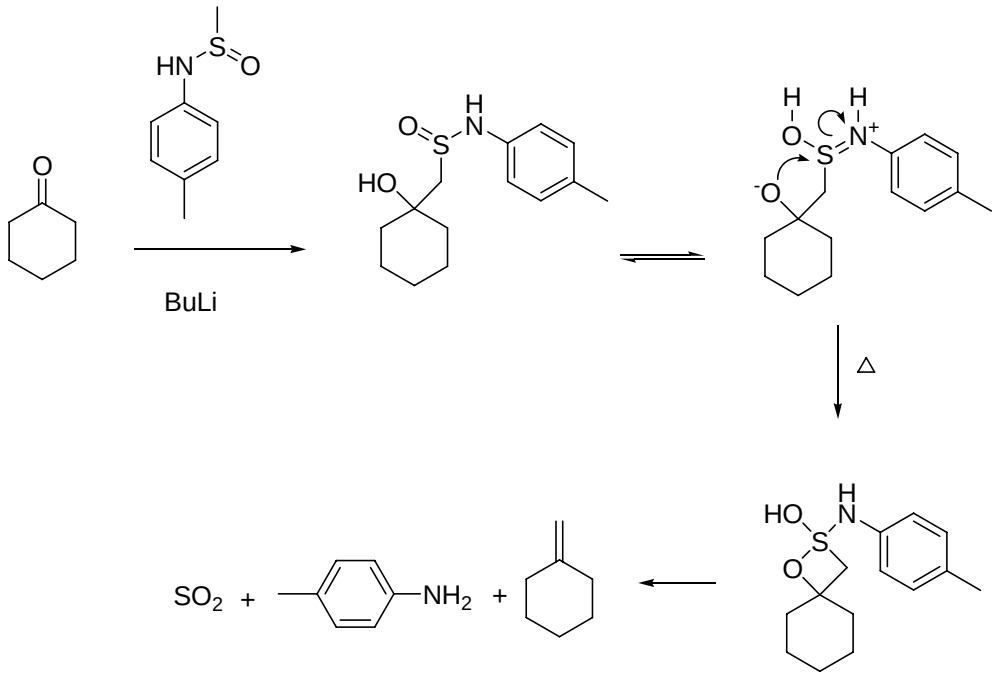
The various methods of carbonyl olefination all incorporate an element which is highly oxophilic. The Wittig reaction uses a phosphorus based system, however silicon, sulfur, titanium and zinc systems are also used. In general an intermediate 4-membered ring (eg the cyclophosphetane in the Wittig reaction) is involved from which the oxidised oxophilic element is subsequently eliminated to yield the olefin. In several cases a stable carbonyl addition product is initially obtained, from which the elimination must be carried out. A brief review of methylenation reactions will be given here.

The Peterson olefination is based on silicon chemistry and involves addition of a silylcarbanion onto the carbonyl (**Scheme 2.34**).[112] The magnesium alkoxides initially formed are too stable to eliminate but exchange of the counter-ion for sodium or potassium leads to smooth elimination. Acidic conditions can also be employed to achieve elimination. The Peterson reaction often gives improved yields compared to the Wittig reaction.



Scheme 2.34 – Example of Peterson olefination

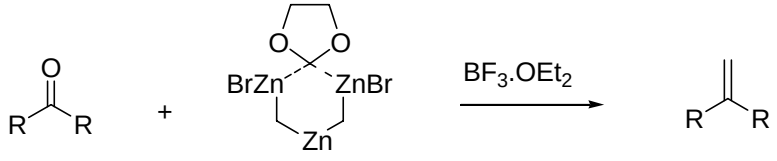
The use of sulfur chemistry in carbonyl olefination was demonstrated by Corey and Durst using sulfinamides.[113] The addition of *N*-methanesulfinyl-*p*-toluidine to a carbonyl yields a β-hydroxy sulfinamide, which eliminates thermally to give an olefin (**Scheme 2.35**). The high basicity of the *N*-methanesulfinyl-*p*-toluidine dianion makes this method inappropriate with enolizable ketones and base sensitive substrates.



Scheme 2.35 – Sulfinamide olefination

The Julia olefination is another sulfur based system, involving addition of a metallated sulfone followed by activation of the resultant alcohol as a mesylate, and reductive elimination using sodium/mercury amalgams to give the olefin. This method is, however, rarely used for methylenation due to the existence of simpler procedures.

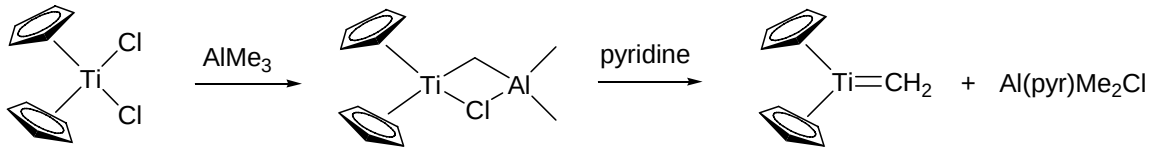
Transition metal based systems have been developed, using zinc and titanium. Nysted's zinc based methylenation reagent, **2.61**, is an example (**Scheme 2.36**).[114] This reagent is particularly useful for enolizable ketones since it requires Lewis acid activation.



2.61

Scheme 2.36 – Nysted's methylenation reagent

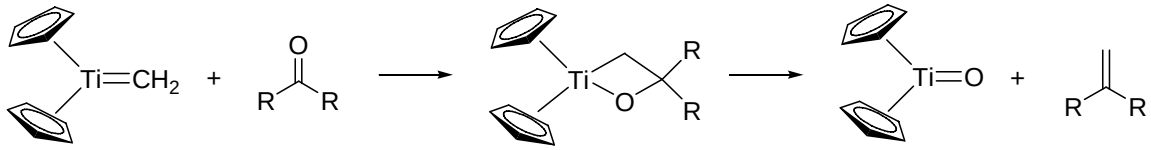
The most important advance in methylenation chemistry came with the discovery of titanium based reagents, combining mild conditions with high yields. The first of these was the Tebbe reagent, **2.62**, prepared from titanocene(IV) dichloride with trimethylaluminium.[115] Treatment of the reagent with a mild Lewis base such as pyridine produces the Schrock carbene, **2.63**, which is the active reagent (**Scheme 2.37**).



2.62 **2.63**

Scheme 2.37 – Generation of the Tebbe reagent and Schrock carbene

In Schrock carbenes, the carbon centre is nucleophilic and the metal centre is electrophilic. Thus this system mirrors that of the phosphorus ylide in the Wittig reaction and the same reactivity is observed, with a strong titanium-oxygen bond eventually formed. Thus, the carbene undergoes a [2+2] cycloaddition with the carbonyl forming an oxatitanocyclobutane which spontaneously decomposes to give the olefin (**Scheme 2.38**).

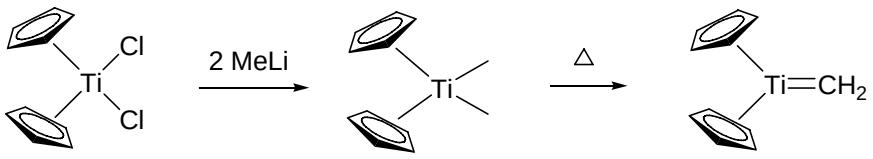


2.63

Scheme 2.38 – Reaction of Schrock carbene with carbonyl

Whilst the Tebbe reagent is a very useful reagent, its significant sensitivity to air and moisture makes it a difficult reagent to handle and it has now been largely displaced by the milder and more stable Petasis reagent, **2.64** (**Scheme 2.39**).[116] This reagent, which is also prepared from titanocene(IV) dichloride, allows access to the same Schrock carbene, **2.63**. It is generated by reaction of titanocene(IV) dichloride with two equivalents of methyl lithium. Upon heating, this complex decomposes to the active Schrock carbene, without releasing a Lewis acidic by-product, as is the case with the Tebbe reagent. As

such, this reagent is particularly mild, methylenating under neutral conditions. Relatively high reaction temperatures are required however, with decomposition to the carbene occurring at around 60-70ºC, and the reagent is sensitive to light.



2.64 **2.63**

Scheme 2.39– Generation of the Petasis reagent and subsequent Schrock carbene formation

One interesting property of the titanium based reagents is an ability to olefinate carboxylic acid derivatives such as esters and amides, although these reactions are slower than reaction at a ketone or aldehyde.

The preceding review suggests that the Petasis reagent is the reagent of choice for methylenating base or acid sensitive substrates. The starting material is inexpensive, the reagent relatively easy to make and handle, and yields are often high.

It was decided to apply this chemistry to the 4-methylproline synthesis in the hope that methylenation could be achieved without racemisation of the α carbon. The methylenation of **2.51** and *ent*-**2.51** was therefore undertaken. Synthesis of the Petasis reagent was carried out by the method described by Ndakala *et al*., in which titanocene dichloride in toluene is reacted with methyl lithium in diethyl ether at 0ºC.[117] The reagent is then purified by extraction with cold ammonium chloride. The solvent is partially removed until a concentrated toluene solution of the reagent is obtained. This is necessary because it is unstable in the solid form. Care is also taken to limit exposure to light throughout the preparation and use of the reagent. The solution of the reagent is then added to the substrate ketone and the reaction heated.

Reaction conditions, and in particular, work-up conditions were optimised such that this reaction achieved a 56% yield on one occasion. This result was not reproducible, however, with yields normally ranging from 30 to 45%. Heating of the ketone with Petasis reagent in toluene at 90ºC for 3 hours were found to be the optimal conditions for reaction. After cooling it was important to add the toluene reaction solution dropwise to a large excess of petroleum ether. In this way, most of the titanium residues could be precipitated, allowing a relatively clean reaction product to be obtained after filtration through Celite. ^{1}H NMR spectroscopy of the crude reaction mixture after 3 hours showed that no starting material remained, however, after work-up and purification of the crude residue by chromatography on silica, the yields were invariably disappointing (values above).

The products obtained from methylenation with the Petasis reagent had identical NMR spectral properties to those obtained from the Wittig reaction. Pleasingly, the two enantiomers now displayed strong optical activity (-26º for **2.52** and +25º for *ent*-**2.52**), suggesting that the integrity at the α carbon had been maintained.

The exomethylene was stereodivergently hydrogenated under the same conditions as discussed earlier and gave the anticipated products, **2.53**, **2.54** and their enantiomers. Both hydrogenation products showed significant optical activity as expected (-43º for **2.53** and +41º for *ent*-**2.53**; -36º for **2.54** and +39º for *ent*-**2.54**). These results do not necessarily indicate optical purity, however. It was evident from the ^{1}H NMR spectra of **2.54** and its enantiomer that small amounts of diastereoisomeric products were present, in a ratio of approximately 7 to 1, however broadening of the peaks made accurate integration difficult (**Figure 2.9**). As previously discussed, trace impurities were present in the ^{1}H NMR spectrum of **2.53** and its enantiomer (**Figure 2.10**), which were tentatively assigned as the diastereoisomeric product. Carbamate isomerisation of the CBz group accounts for the doubling of peaks observed in the ^{1}H NMR spectrum of **2.53**.

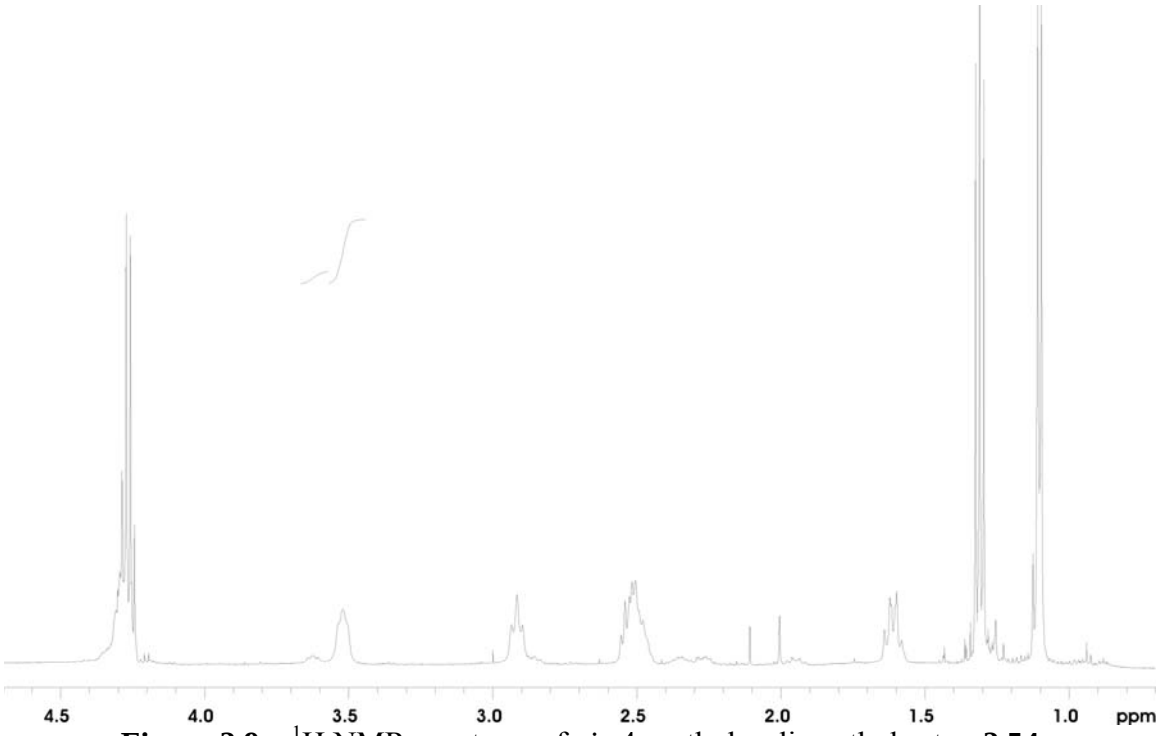


Figure 2.9 – ^{1}H NMR spectrum of *cis*-4-methylproline ethyl ester, **2.54**

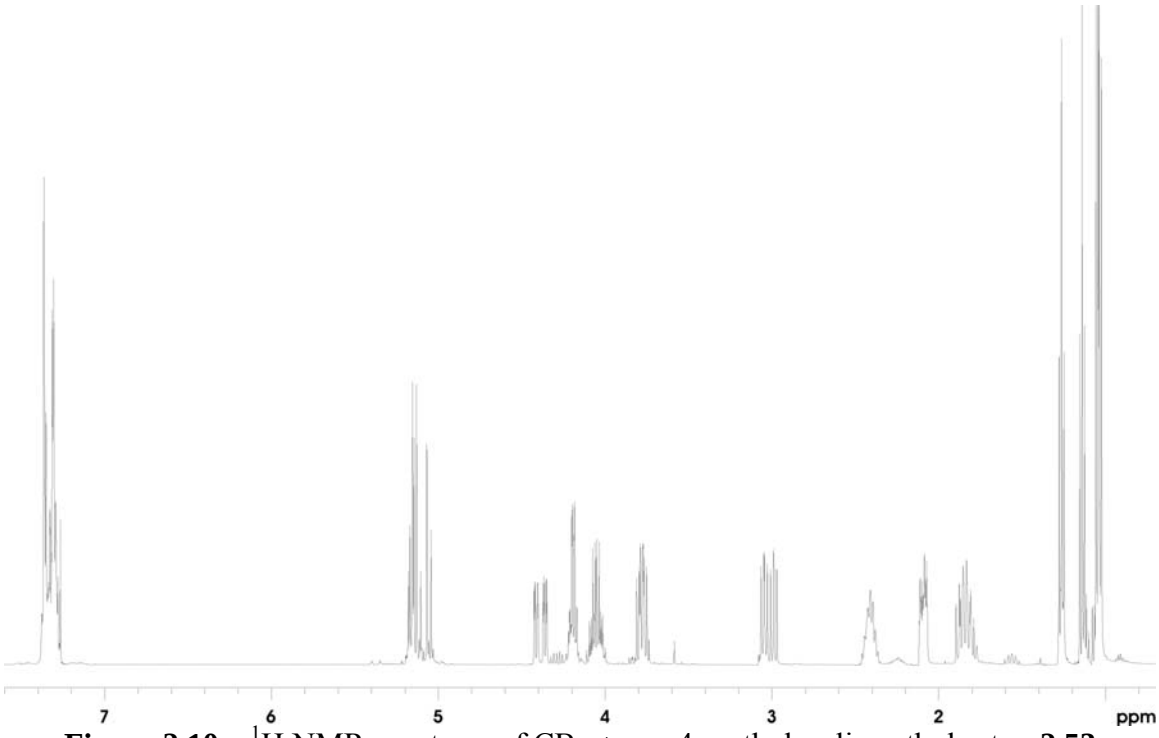


Figure 2.10 – ^{1}H NMR spectrum of CBz-*trans*-4-methylproline ethyl ester, **2.53**

These results suggested that the two hydrogenation procedures had occurred with a reasonable stereoselectivity, but not in a totally stereospecific manner. This postulate was examined more closely once HPLC of the Marfey's derivatives of the final products was carried out (*vide infra*).

The mode of cleavage of the remaining protecting groups on the 4-methylproline derivatives (CBz and ester in the case of **2.53** and ester in the case of **2.54**) was adapted in order to abbreviate the route. Acid hydrolysis would be capable of removing both the CBz and the ester of **2.53** where previously hydrogenation to remove the CBz was followed by a basic hydrolysis to cleave the ester. In addition, the use of $HCl_{(aq)}$ would allow accurate measurement of the optical rotation of the HCl salt to be carried out, since any excess could be readily removed under vacuum. Finally, the use of acidic conditions for protecting group cleavage would be expected to occur without racemisation, since amino acids are generally only sensitive to basic conditions.

Acid hydrolysis was carried out by heating the amino acid derivatives, **2.53**, **2.54** and their enantiomers, in aqueous HCl (6 M) at 110ºC overnight. This successfully cleaved all protecting groups, although ^{1}H NMR spectroscopy (**Figure 2.11**) of the 4-methylproline products suggested that diastereoisomeric mixtures had been formed. The presence of a reasonably significant proportion of the undesired diastereoisomer appeared to be present, of the order of around 3 to 1 in both cases. ^{1}H NMR spectroscopy of **2.53** and **2.54** (**Figures 2.10** and **2.9** respectively) had suggested a greater stereoselectivity in both hydrogenations, so this result was puzzling.

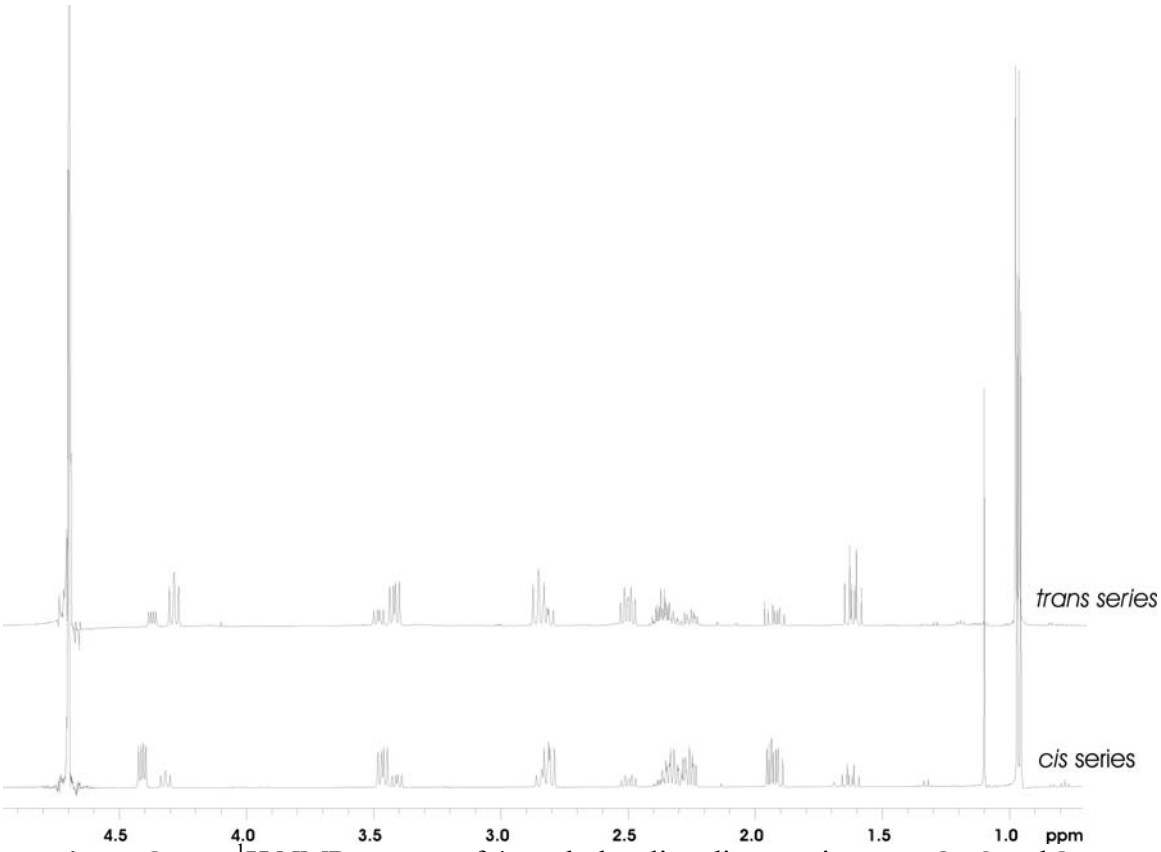


Figure 2.11 – ^{1}H NMR spectra of 4-methylproline diastereoisomers, **2.56** and **2.57**

The four diastereoisomeric mixtures were derivatised with Marfey's reagent and analysed by HPLC (**Figure 2.12**).

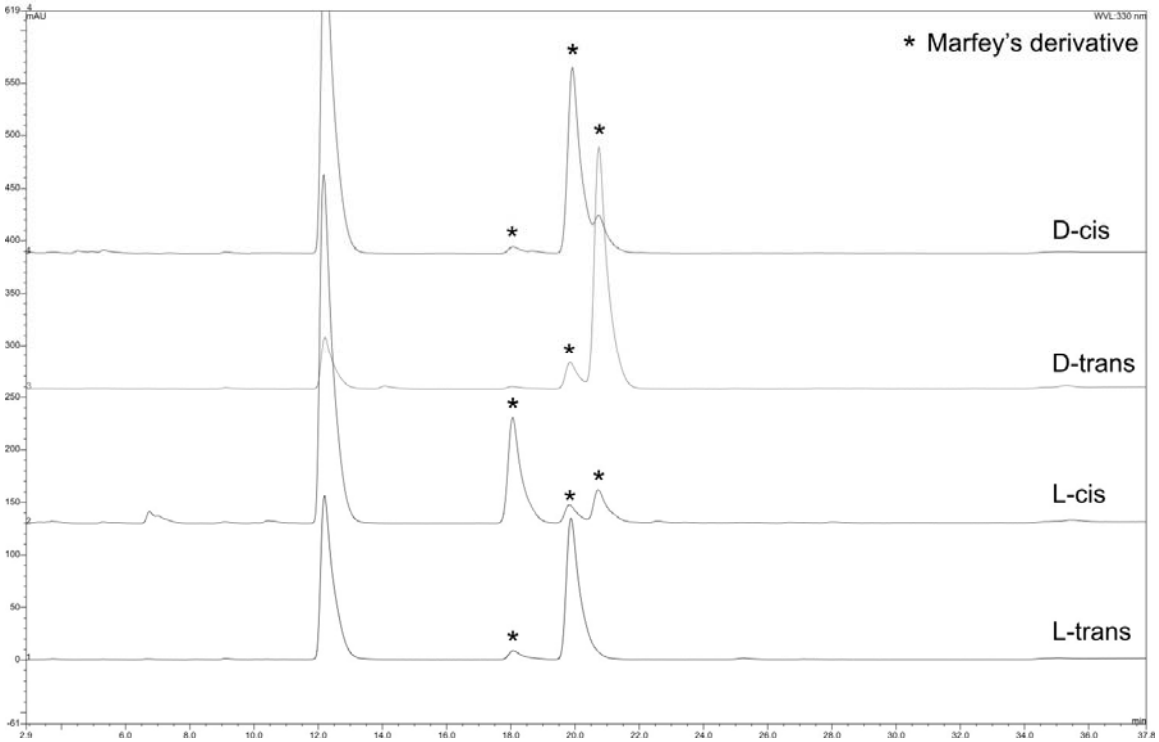


Figure 2.12 – HPLC of Marfey's derivatives of 4-methylproline synthesised by Petasis methylenation route

These HPLC results indicated that epimerisation of the α carbon might have been the cause of the disappointing diastereoisomeric mixtures observed by ^{1}H NMR spectroscopy. This conclusion was drawn due principally to the evidence given by the HPLC chromatogram of *cis*-4-methyl-L-proline. Three Marfey's derivative peaks are in evidence, the major peak being that of the L-*cis* diastereoisomer. Due to the lack of stereospecificity during the hydrogenation step, a peak for the L-*trans* diastereomer was also expected, and was present in a ratio of around 1:7 to the major peak. However, the final peak corresponds to the D-*trans* diastereoisomer and was present in around a 1:3 ratio with the major peak. This represents epimerisation at the α carbon of the L-*cis* major diastereoisomer. It is possible that the product of racemisation of the minor L-*trans* product (D-*cis*) was also present, however the resolution obtained between D-*cis* and L-*trans* isomers was not sufficient to confirm this. Optimisation of HPLC conditions was attempted to improve the resolution of these residues, however no significant improvement could be made.

The HPLC chromatogram of *cis*-4-methyl-L-proline could not be fully analysed, again due to a lack of resolution between D-*cis* and L-*trans* under these conditions. Analysis of the HPLC chromatograms of the two *trans*- diastereoisomers was somewhat confusing, however. Only two peaks were present in each, and the minor peak (present in a ratio of around 1:15) in each case, was the expected *cis*- diastereoisomer, formed due to lack of stereospecificity during the hydrogenation stage. This was precisely the result hoped for, however it was not the result anticipated from analysis of the ^{1}H NMR spectrum (**Figure 2.11**).

In order to examine whether acid hydrolysis was leading to the racemisation of the α carbon, Marfey's derivatisation was carried out on **2.54**, the compound immediately prior to the acid hydrolysis. The *cis*-4-methyl-L-proline ethyl ester, **2.54**, was chosen as it would allow direct comparison to the HPLC results above which clearly indicated α carbon racemisation. The HPLC results are shown in **Figure 2.13**. It can be seen that only two diastereoisomers are present, compared with the three observed after acid hydrolysis. This lent further credence to the postulate that acid hydrolysis was causing α carbon racemisation.

The harsh conditions of acid hydrolysis were therefore optimised. It was found that complete cleavage of both protecting groups could be achieved at 70ºC in 6 hours. The ^{1}H NMR of the hydrolysis products were significantly cleaner, containing only trace amounts of diastereoisomeric impurities (**Figure 2.14**).

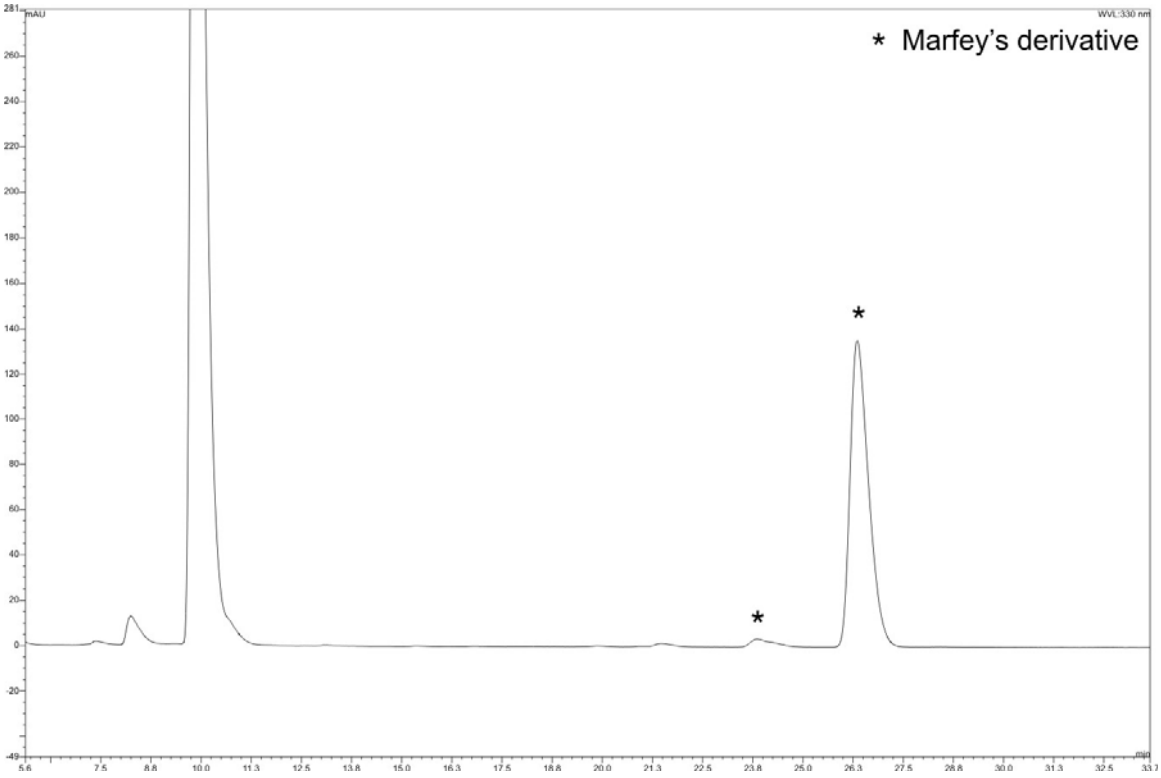


Figure 2.13 – HPLC analysis of the Marfey's derivative of *cis*-4-methyl-L-proline ethyl ester

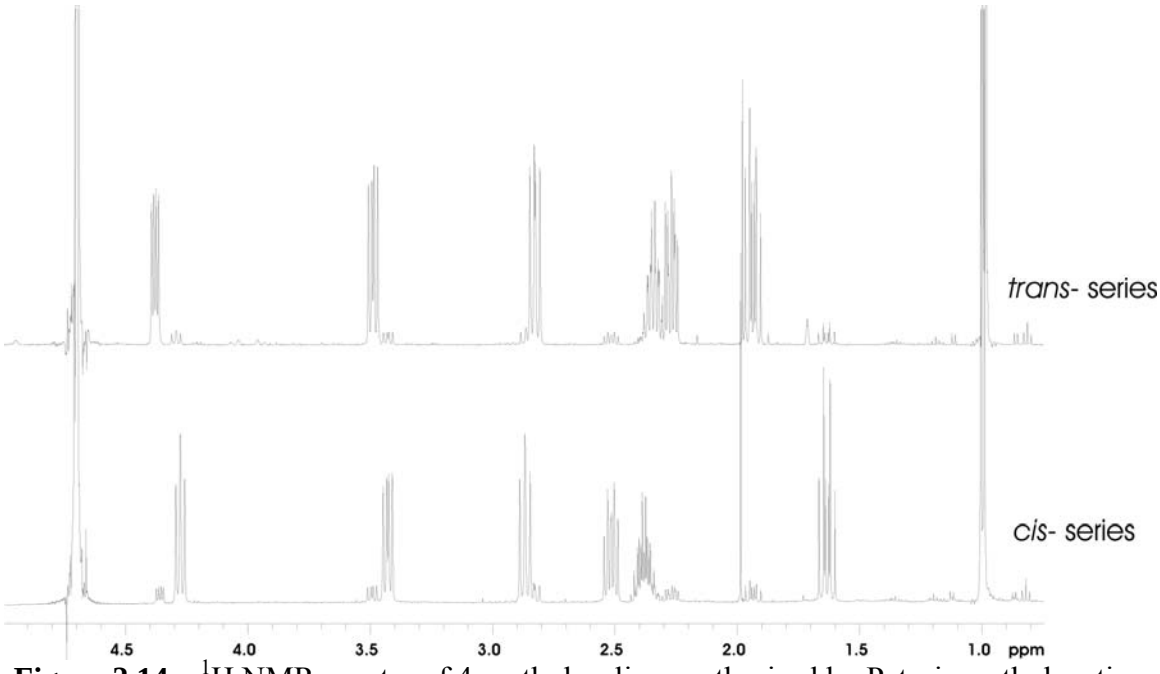


Figure 2.14 – ^{1}H NMR spectra of 4-methylproline synthesised by Petasis methylenation route employing milder deprotection method

Marfey's derivatisation of the 4-methylproline residues and analysis by HPLC (**Figure 2.15**) showed that the levels of racemisation had been significantly reduced, although not entirely eliminated. Thus, examination of the chromatogram of the Marfey's derivative of the L-*cis* diastereoisomer revealed a ratio of 15:1 of the major peak to the product of racemisation at the α carbon (D-*trans*). The diastereoisomeric impurity derived from the lack of stereospecificity of the hydrogenation (L-*trans*) was present in a ratio of 1:7 of the major peak.

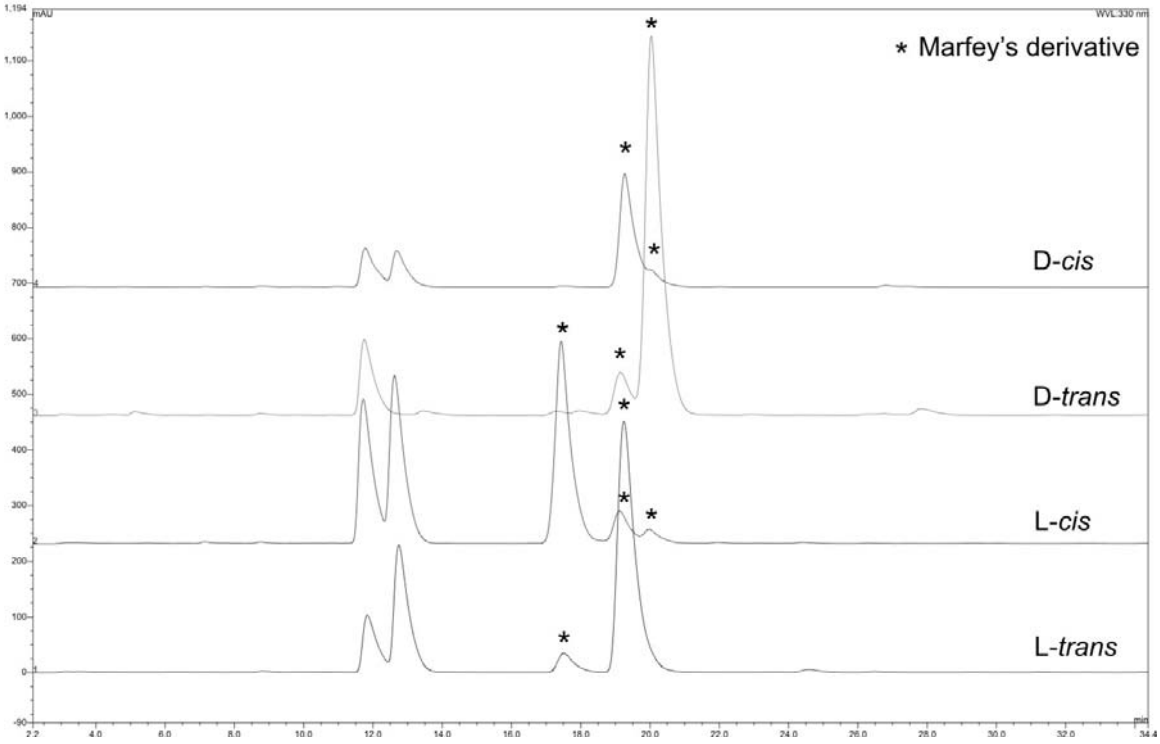


Figure 2.15 - HPLC of Marfey's derivatives of 4-methylproline synthesised by Petasis methylenation route employing milder deprotection method

Examination of the chromatograms of the other three diastereoisomers of 4-methylproline also revealed relatively good diastereoisomeric purity. Exact diastereoisomeric ratios could not be calculated for *cis*-4-Methyl-D-proline due to an overlap of the major peak with the minor D-*trans* diastereoisomer, however visual analysis suggested similar levels to those seen for L-*cis*. The *trans*- series showed a ratio of around 15:1 of the major isomer to the minor.

The stereoselectivity of the stereodivergent hydrogenations could now be analysed. The stereoselectivity observed in the heterogeneous hydrogenation using palladium on carbon was 7 to 1. This is not as high as that reported by Moody *et al*., however it is significantly higher than that observed by Del Valle and Goodman. This latter result may be rationalised by considering the steric bulk imposed by the ester functional group, hindering approach of the substrate to the catalyst on that face. Whilst bulky, the protected alcohol group which fulfills the same role in Del Valle's synthesis would be expected to be more flexible, since the C1 carbon (sp^3) is a primary alcohol. With free rotation between C1, C2 and the protected alcohol, the bulky silyl group can adopt a position which does not significantly hinder the substrate's approach to a surface. In the case of the ester, C1 (sp^2) is surrounded by two bulky oxygen atoms, steric bulk which rotation about the C1 to C2 bond does not alleviate. The protected alcohol therefore permits approach on the same face more readily than the ester.

The stereoselectivity observed with Crabtree's homogeneous hydrogenation was 15 to 1. This is significantly lower than the stereoselectivity observed by Del Valle and Goodman, who obtained ratios of greater than 40 to 1. This lower stereoselectivity can be rationalised by comparing the efficiency of the directing groups used to steer the hydrogenation onto one face. Alcohols are excellent directing groups, due to the ready availability of their lone pair to coordinate to the metal centre and their minimal steric bulk. On the other hand, whilst esters have been shown to be good directing groups, they are known to be less efficient in this role than alcohols.[104] This is because their lone pair is less available to the metal both due to resonance within the ester, and to a lesser degree, due to the bulkiness of the ester impeding approach of the metal to the lone pair. With the directing effect slightly diminished, competing, non-directed hydrogenations take place, leading to a lowering of the observed stereoselectivity.

2.3.4 Epimerisation of 4-hydroxyproline

In order to extend the utility of this synthetic route to 4-methylproline, the reported epimerisation of *trans*-4-hydroxy-L-proline to give *cis*-4-hydroxy-D-proline was examined. If the D- series could be readily accessed from the inexpensive *trans*-4-hydroxy-L-proline, this route would allow all four diastereoisomers to be generated from a single, readily available starting material.

The epimerisation of *trans*-4-hydroxy-L-proline was first reported by Baker *et al*. and, according to the authors, leads to complete inversion of the α carbon stereochemistry.[118] This method has subsequently been employed by other groups,[119,120] and most notably by Heindl and co-workers during their synthesis of 4-substituted prolines, discussed earlier.[84]

Therefore the epimerisation of *trans*-4-hydroxy-L-proline following the method of Baker *et al* was attempted. It is a simple two-step protocol, which does not require purification of the intermediate. The starting material was first refluxed in acetic anhydride and glacial acetic acid, then after removal of these reagents *in vacuo*, the residue was taken up in 2M HCl(aq) and further refluxed. After filtration, the product was obtained by simple removal of all solvents and reagents to give the product as a crystalline solid. This protocol gave the product as a slightly off-white solid. ^{1}H NMR spectroscopy of the product revealed, however, that epimerisation of the α carbon had not been complete (**Figure 2.16**). Small changes in chemical shift were observed. This was due to the fact that the product of the epimerisation reaction yields the HCl salt, whereas the commercial amino acids were present as the free amino acid.

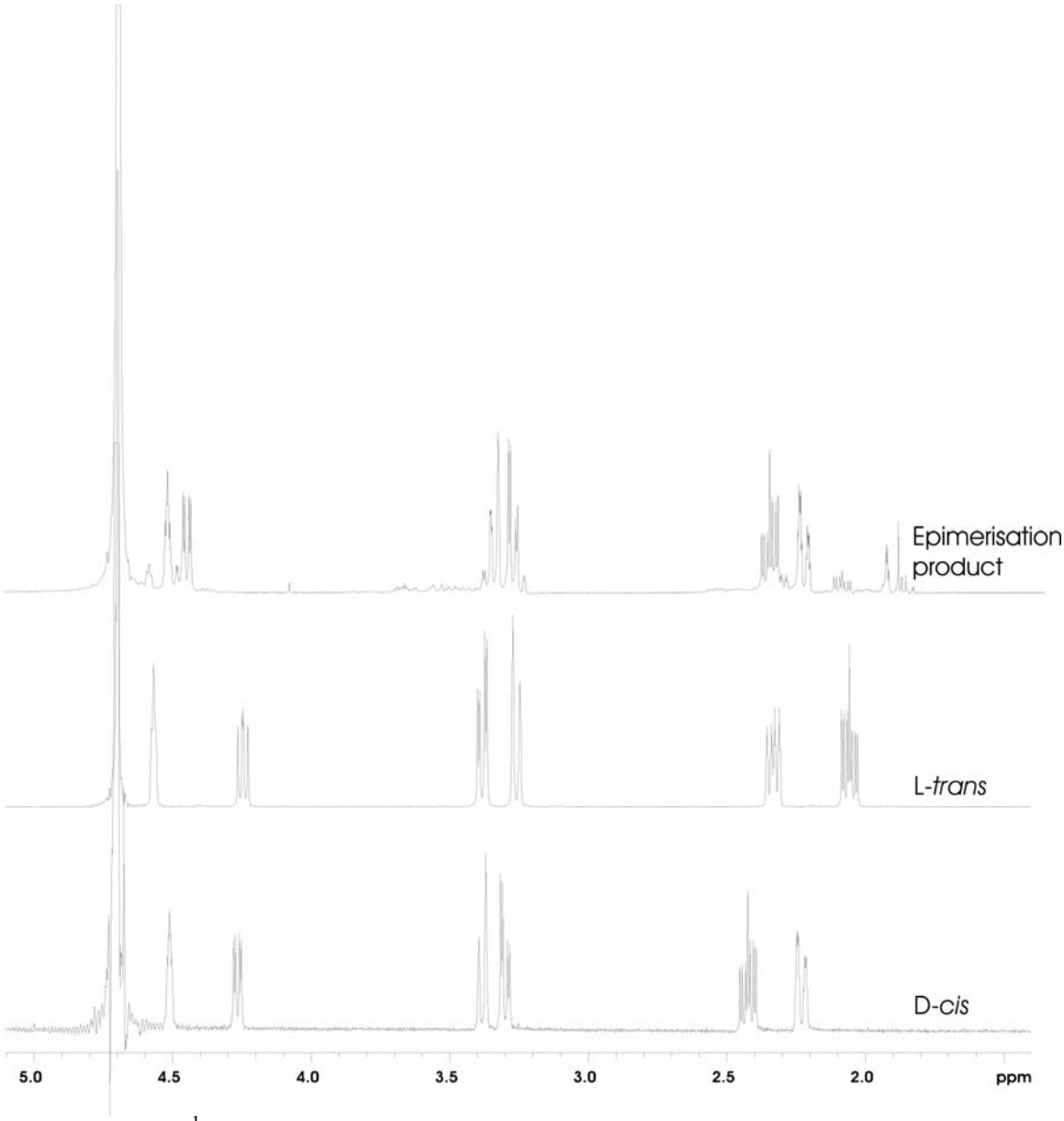


Figure 2.16 – ^{1}H NMR spectra of the product of epimerisation and commercial L-*trans* and D-*cis* hydroxyproline

In order to confirm the stereochemical outcome of the reaction, a comparison of the Marfey's derivatives of the four commercial diastereoisomers of 4-hydroxyproline and the reaction product was made. The results of HPLC analysis of the derivatives is shown in **Figure 2.17**.

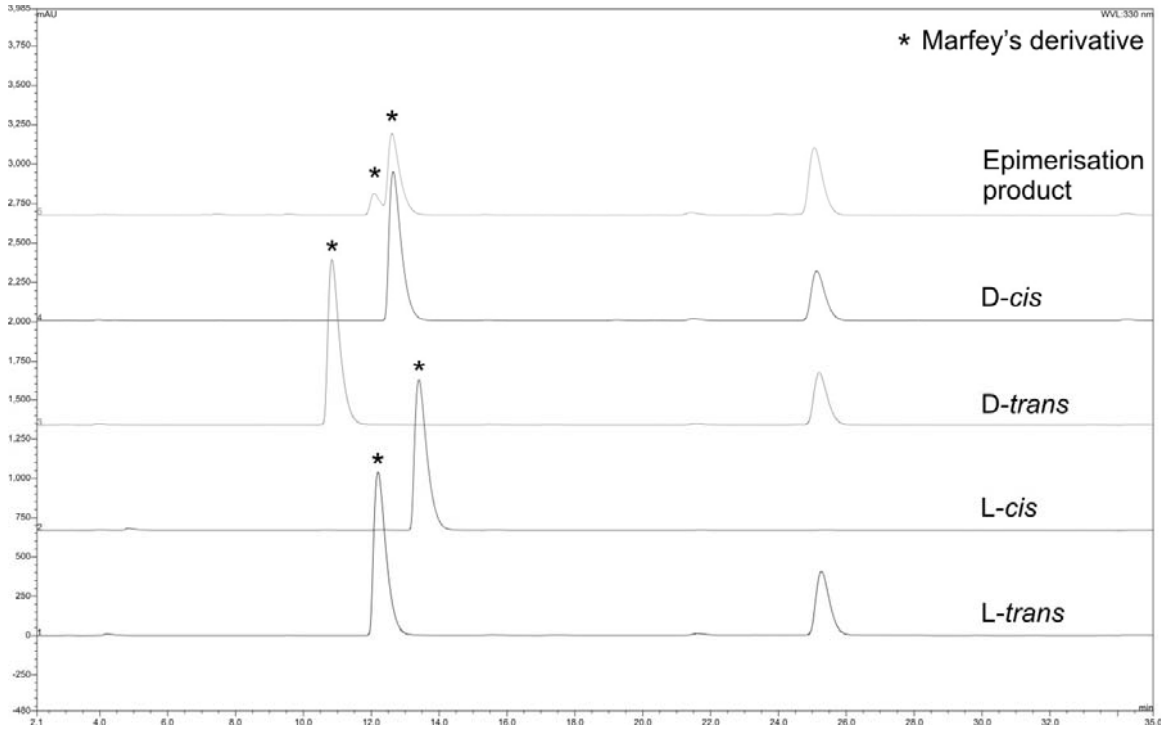


Figure 2.17 – HPLC analysis of the Marfey's derivative of the product of epimerisation and the four commercial diastereoisomers of hydroxyproline

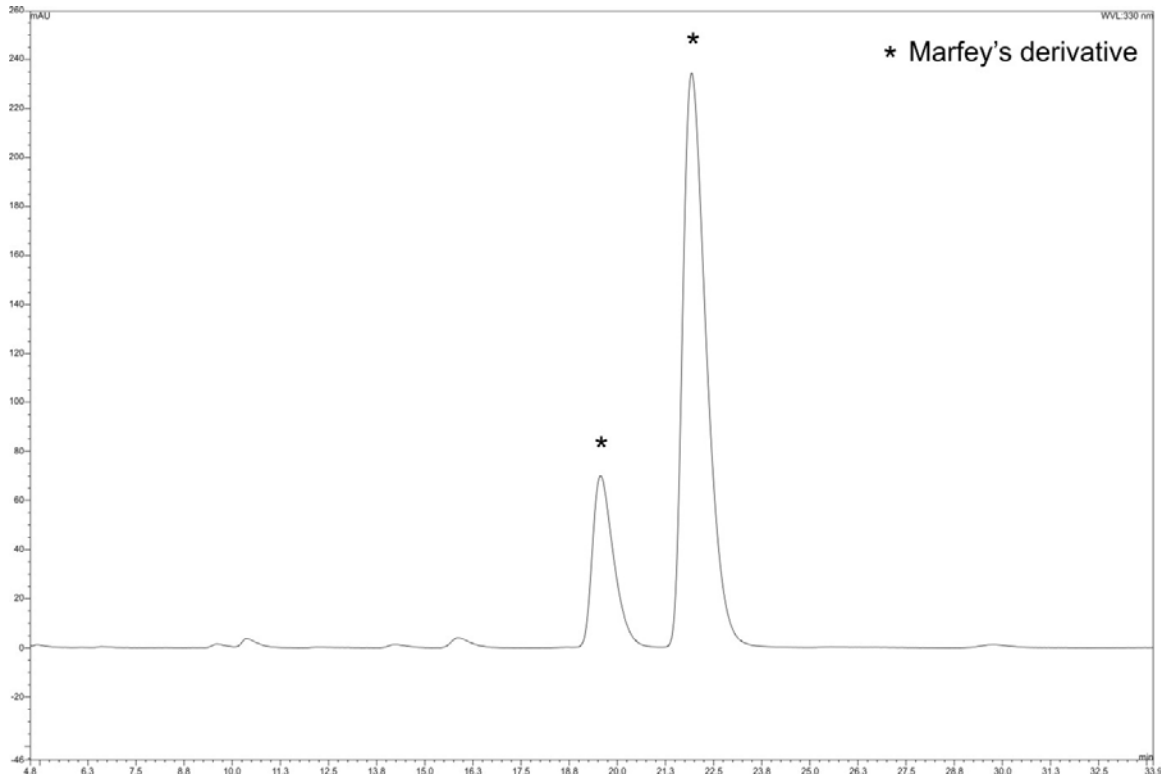


Figure 2.18 – HPLC of the resolved Marfey's derivatives of the product of epimerisation

HPLC analysis clearly shows that whilst D-*cis* is the major diastereoisomeric product of the epimerisation method, a small amount of the starting L-*trans* diastereoisomer remains. To ascertain the exact ratio of product to starting material, optimisation of the HPLC conditions was undertaken, since the two peaks were not resolved. The use of an optimised HPLC method are shown in **Figure 2.18**, and allowed the ratio of major to minor peak to be estimated at 4 to 1.

No attempts to optimise the reaction conditions of this epimerisation to improve the stereoselectivity were undertaken. It is evident that this literature procedure does allow ready access to the D- series of hydroxyproline, and therefore allows the synthesis of all four diastereoisomer of 4-methylproline from one starting material. However, it is does not occur in an entirely stereospecific manner (as reported) and therefore should only be used where high levels of optical purity in the final products are not required. In the case of those undertaking the assignment of a naturally occurring 4-methylproline in a secondary metabolite, less vigorous levels of optical purity are probably acceptable.

2.3.5 Conclusions

By this route all four diastereoisomers of 4-methylproline have been successfully synthesised, with a good level of stereoselectivity, in just six steps from two inexpensive starting materials. The yields at most steps were high, in the range of 80 to 100%, however the yields obtained during the formation of the exomethylene were consistently low, with a maximum isolated yield of 56%. This detracted from the maximum cumulative yields, which were 46% for the *cis*- series and 34% for the *trans*- series. The stereoselectivities observed were of the order of 7 to 1 for the *cis*- series and 15 to 1 for the *trans*- series. It has also been shown that the inclusion of one (two-part) additional step into the synthesis would allow all four diastereoisomers to be obtained from just one starting material, although this would be accompanied by a small decrease in product optical purity.

This route compares favourably to the route reported by Del Valle and Goodman. Whilst their route may be more applicable when synthesising 4-substituted prolinols, this method provides more succinct access to 4-methylproline itself. The route of Del Valle and Goodman is 8 steps, although the authors did not remove the Boc protecting group, so that it becomes 9 steps if the fully deprotected amino acid is required. The route developed here compares favourably, with only 6 steps to final deprotected 4-methylproline. The cumulative yield of this route is better than that obtained by Del Valle and Goodman, although direct comparison is difficult as they did not fully deprotect the product and only carried out the penultimate oxidation on the *trans*- methyl derivative. They reported a 26% cumulative yield for the Boc-*trans*-4-methylproline and 28% for the Boc-*cis*-4-methylprolinol. In addition, this route offers more consistent stereoselectivity to the *cis*- and *trans*- series. Del Valle and Goodman observed a ratio of greater than 40 to 1 for the *trans*- series (compared to 15 to 1), however they obtained a maximum of 3 to 1 for the *cis*- series (compared to 7 to 1).

The route also compares favourably to the other principal stereosepecific route to protected 4-methylproline derivatives, reported by Heindl *et al*. Whilst Heindl's route is stereospecific, it suffers from low cumulative yields (44% for *N*-benzyl-*trans*-4-methyl-D-proline and 9% for *N*-benzyl-*cis*-4-methyl-L-proline ethyl ester) and does not report the potentially difficult deprotection of the *N*-benzyl group. Also it includes the use of cuprate reagents which proved to be unreliable in the present work.

Therefore overall this route offers an improvement on the literature procedures for synthesising 4-methylproline, a naturally-occurring and pharmaceutically relevant amino acid.

2.4 Stereochemical assignment of the pteratides

With all of the reference amino acids in hand, it was possible to undertake the full stereochemical elucidation of the pteratides. Hydrolysis of each of the depsipeptides was carried out under acidic conditions to give the free amino acid mixture. Marfey's derivatisation of all reference amino acids and each of the pteratide hydrolysates was then carried out. Finally, HPLC analysis was carried out to examine which amino acids were present in the hydrolysates. Good resolution of the stereoisomers of each amino acid was important for the accurate assignment of the amino acids present in the natural product hydrolysate. In addition, good resolution of the constituent amino acids in the natural product hydrolysate mixture was taken into account when optimising HPLC elution conditions. Two different methods were therefore used for the HPLC analyses, in order to obtain good resolution of the each of the amino acids. In the case of pteratide I, several of the amino acids (phenylalanine, *N*-methylvaline and *N*-methylleucine) were analysed on a different HPLC instrument (Shimadzu, indicated on appropriate figures), using a similar column and method to that employed on all other occasions. These stereochemical elucidation studies were carried out with the help of Drs Maya Mitova and Gerhard Lang (in particular, the derivatisation of the hydrolysates of pteratides II and III and some of the reference amino acids). The derivatisation of pteratide I and all other reference amino acids was carried out as part of this thesis work.

Examples of the HPLC results for Marfey's derivatives of the commercial reference amino acid residues are given in **Figures 2.19** and **2.20**.

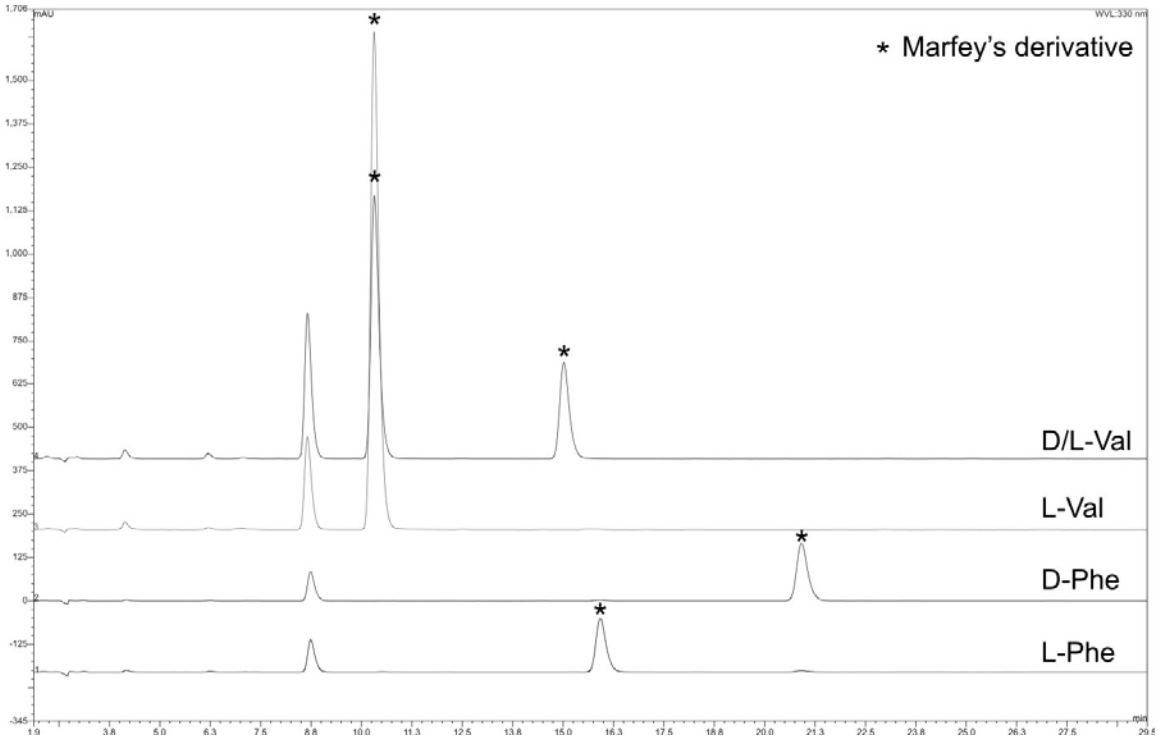


Figure 2.19 – HPLC chromatograms for the Marfey's derivatives of phenylalanine and valine reference amino acids

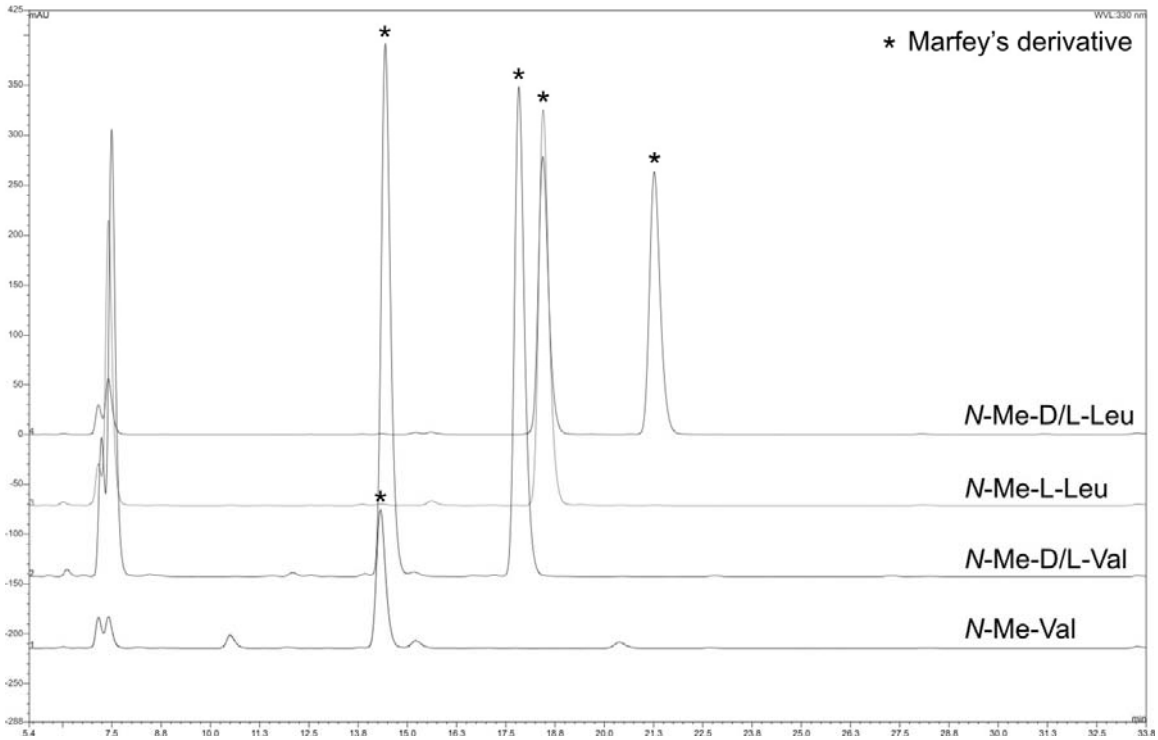


Figure 2.20 - HPLC chromatograms for the Marfey's derivatives of *N*-methylvaline and *N*-methylleucine reference amino acids

The HPLC chromatograms of the Marfey's derivatised hydrolysate products of the pteratides are shown in **Figures 2.21** to **2.25** with the assigned amino acids labelled.

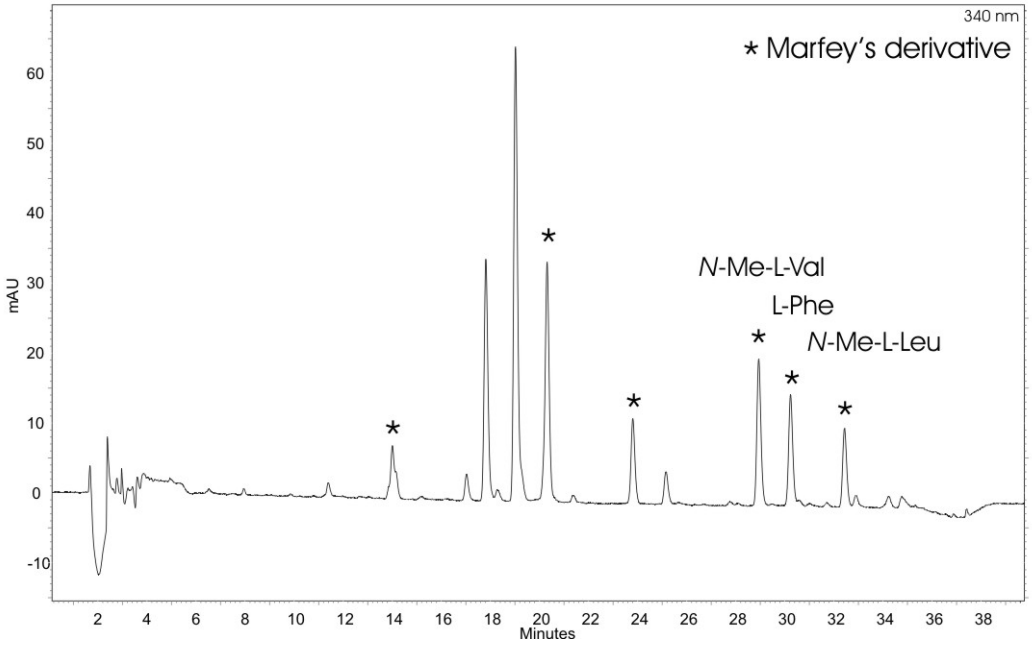


Figure 2.21 – HPLC analysis of the Marfey's derivative of Pteratide I (Shimadzu)

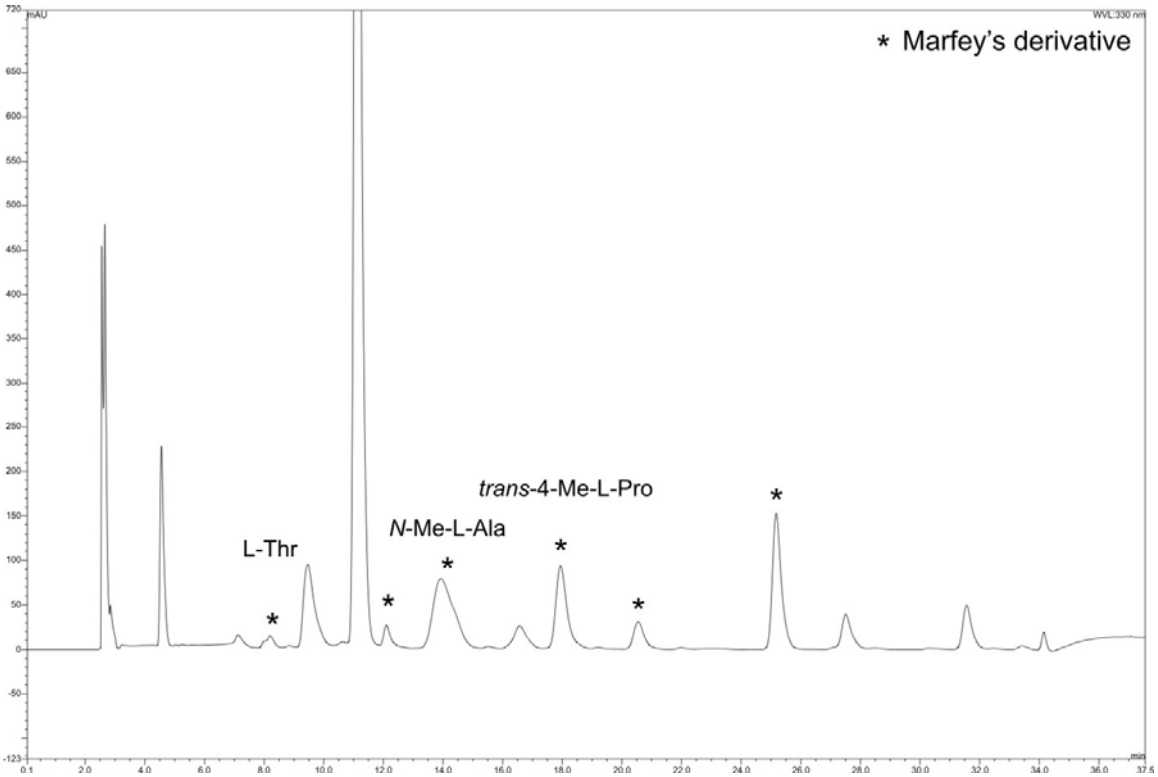


Figure 2.22 - HPLC analysis of the Marfey's derivative of Pteratide I

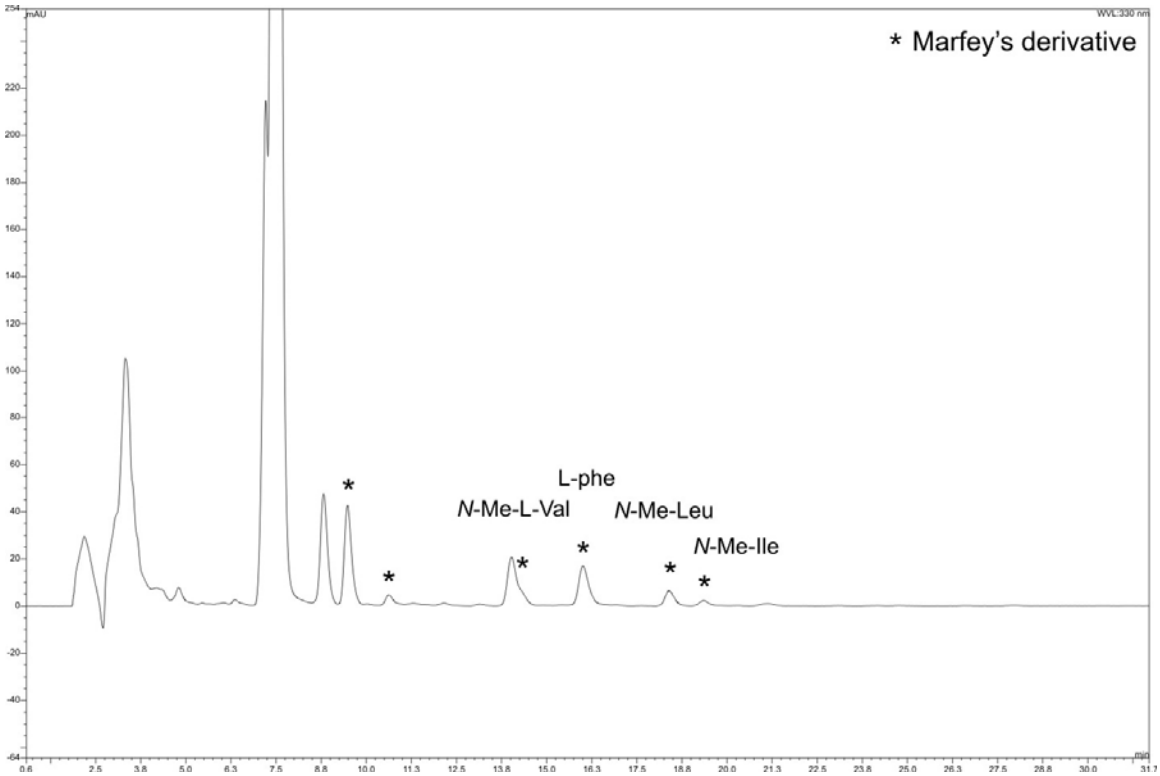


Figure 2.23- HPLC analysis of the Marfey's derivative of Pteratide II

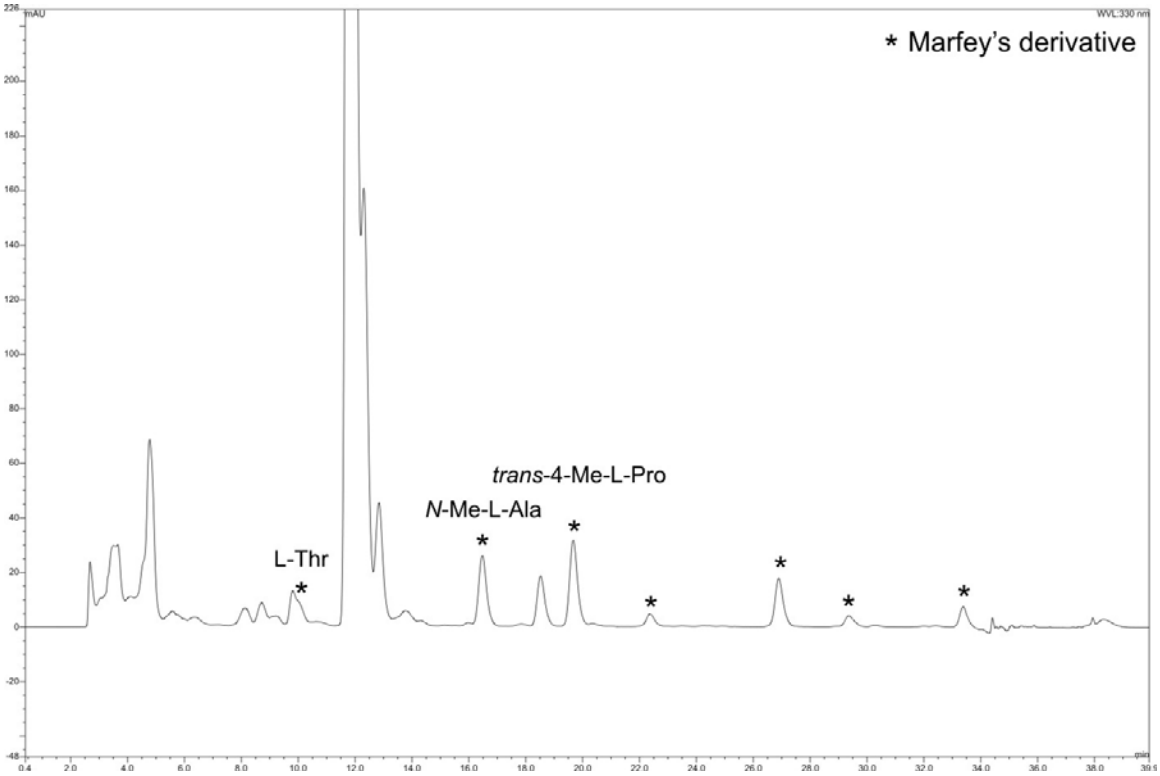


Figure 2.24 – HPLC analysis of the Marfey's derivative of Pteratide II

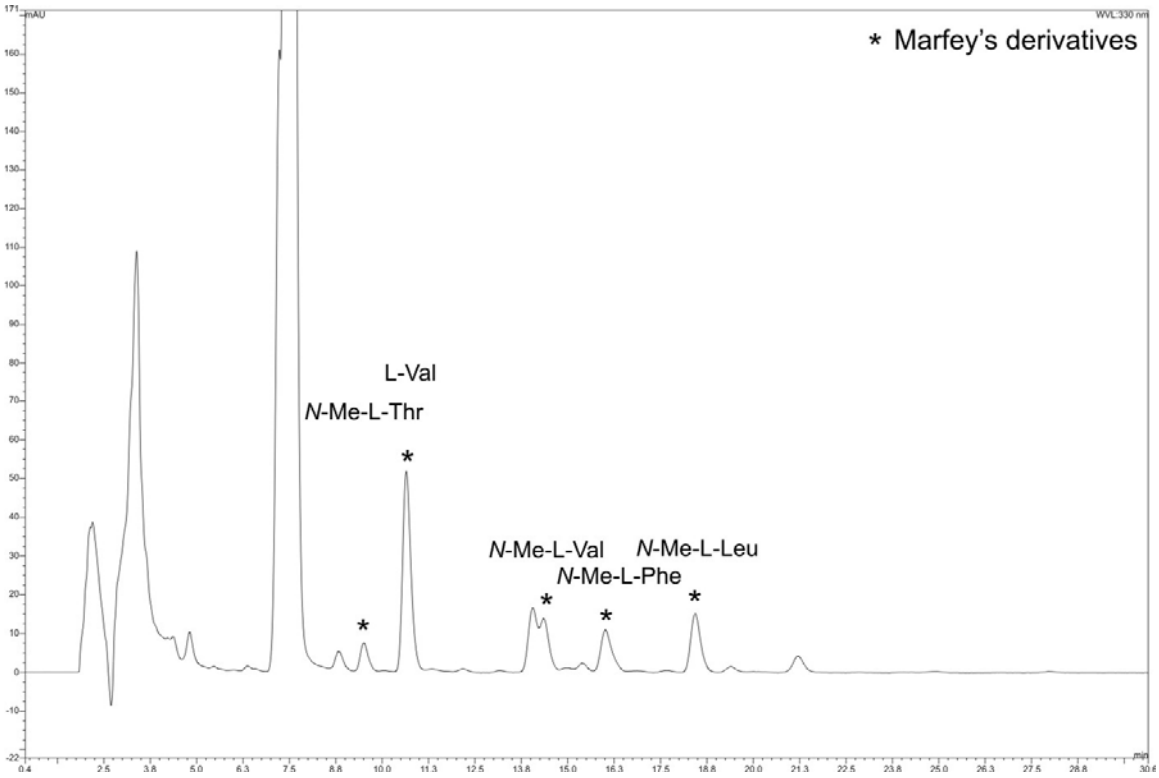


Figure 2.25 – HPLC analysis of the Marfey's derivatives of Pteratide III

Since the Marfey's derivatives of the reference amino acids and pteratide hydrolysates were not run simultaneously on HPLC, a slight drift of the retention time of the hydrolysed Marfey's reagent was observed. The retention times for the amino acids were therefore standardised using the hydrolysed Marfey's reagent as an internal standard. **Table 2.3** shows the standardised retention times for the amino acid standards used to assign the stereochemistry of the pteratides. It also indicates the amino acid which was found to be present in the natural products, by comparison of HPLC retention times.

Table 2.3 – Retention times of amino acid standards and natural product hydrolysates

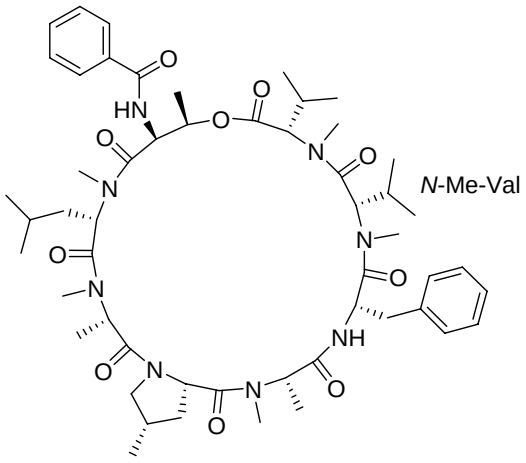
	R_t of standards (min)			pteratide		
Amino acid	Eluent A†	Eluent B†	Shimadzu†	I	II	III
L-valine	10.3					*
D-valine	15.0					
L-threonine		8.9		*	*	
D-threonine		12.9				
L-*allo*-threonine		10.2				
D-*allo*-threonine		10.9				
L-phenylalanine	15.7		30.3	*	*	
D-phenylalanine	20.9		34.1			
N-methyl- L-alanine		15.4		*	*	
N-methyl- D-alanine		15.0				
N-methyl- L-valine	14.3		28.9	*	*	*
N-methyl- D-valine	17.8		31.6			
N-methyl- L-leucine	18.4		32.4	*	*	*
N-methyl- D-leucine	21.3		34.1			
N-methyl- L-isoleucine	19.4				*	
N-methyl- D-isoleucine	24.2					
N-methyl- L-*allo*-isoleucine	19.9					
N-methyl- D-*allo*-isoleucine	22.6					
N-methyl- L-threonine		9.9				*
N-methyl- D-threonine		10.6				
N-methyl- L-*allo*-threonine		15.4				
N-methyl- D-*allo*-threonine		16.0				
N-methyl- L-phenylalanine	16.0					*
N-methyl- D-phenylalanine	16.7					
trans-4-methyl- L-proline		18.3		*	*	
cis-4-methyl- L-proline		16.6				
trans-4-methyl- D-proline		19.3				
cis-4-methyl- D-proline		18.5				

† Elution conditions, including column type, temperature and solvent systems are given on p258-9 (Chapter 5)

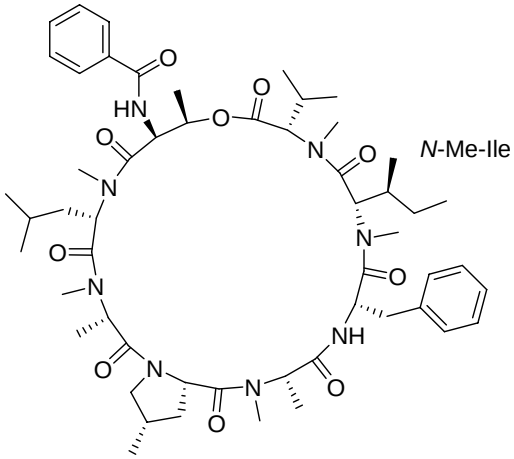
The amino acids were all found to belong to the L- series (*S*) and threonine and isoleucine derived residues were all of the natural stereochemistry at the β position (*R* for threonine, *S* for isoleucine). The 4-methylproline residue was found to have L-*trans*-stereochemistry (2*S*,4*R*).

The structures of the pteratides including their newly assigned stereochemistry are shown below.

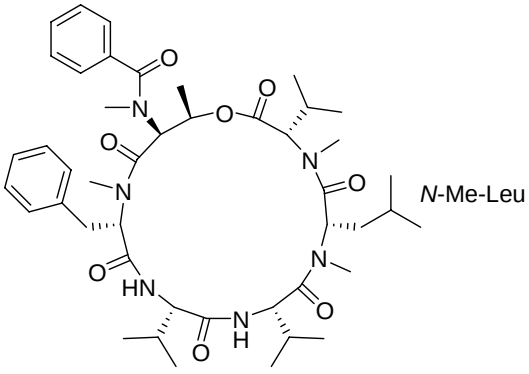
This full structural characterisation of the pteratide structures has been published in the Journal of Organic Chemistry (included in Appendix).[3]



Pteratide I



Pteratide II



Pteratide III

2.5 Final concluding remarks

The syntheses of all possible diastereoisomers of a number of unusual amino acids were successfully completed, using either literature methods or new synthetic routes. These were synthesised with high levels of optical purity. The classical method for stereochemical determination of amino acids was then employed to elucidate the stereochemistry of three naturally occurring depsipeptides.[25] This involved derivatisation of both synthetic and commercially available amino acid standards, and hydrolysates of the natural products, followed by HPLC analysis of the derivatives. The stereochemistry of the natural products were successfully determined, allowing the publication of the full structures of these novel and highly cytotoxic secondary metabolites in a peer-reviewed publication.[3]

2.6 References for chapter 2

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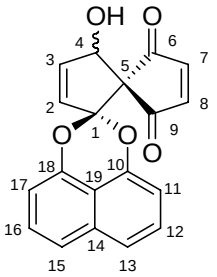
Chapter 3

The *spiro*-Mamakones

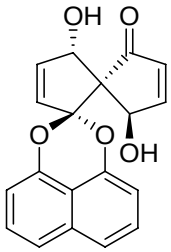
3.1 Introduction

3.1.1 spiro-*Mamakones – new spirobisnaphthalene natural products*

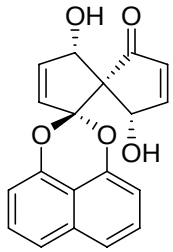
A new series of polyketide derived natural products, the *spiro*-mamakones, **3.1** to **3.5**, were recently discovered in the University of Canterbury natural products group, by Dr S. van der Sar. Of these, *spiro*-mamakone A, **3.1**, has been disclosed in the peer-reviewed literature.[1] These natural products were isolated from the extract of a cultured non-sporulating fungal endophyte derived from the New Zealand native tree *Knightia excelsa* (rewarewa).



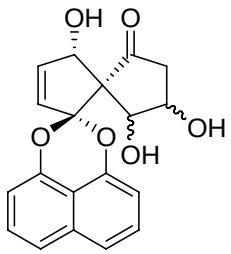
3.1
spiro-mamakone A
(racemic)



3.2
spiro-mamakone B



3.3
spiro-mamakone C

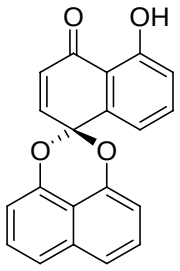


3.4 and **3.5**
spiro-mamakone D and E
(diastereoisomers, stereochemistry unknown)

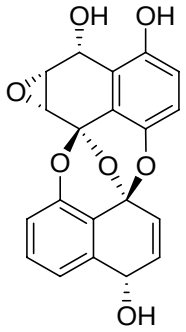
The *spiro*-mamakone series represents a new category in the diverse spirobisnaphthalene class of natural products. Spirobisnaphthalenes are a relatively new class of compounds, with the first example, antibiotic MK3018, appearing in a Japanese patent in 1989 and preussomerin A (**3.7**) appearing in the wider literature in 1990.[2,3] Since then, however, a large number have been found, principally from fungi. Reports have been made of their isolation from plant sources, however the possibility that these plant derived

spirobisnaphthalenes are actually produced by an endophytic fungal source is the subject of some debate.[4,5]

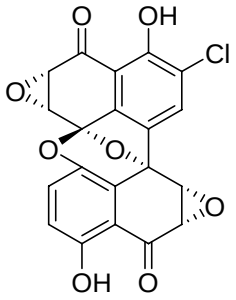
Spirobisnaphthalenes are biosynthesised by the oxidative coupling of two oxygenated naphthalene units. The natural products isolated fall into three general structural types; those with two oxygen bridges between the two naphthalene units, such as palmarumycin CP_1 (**3.6**);[6] those with three oxygen bridges, such as preussomerin A (**3.7**);[3] and those with two oxygen and one carbon bridge, such as spiroxin A (**3.8**).[7]



3.6

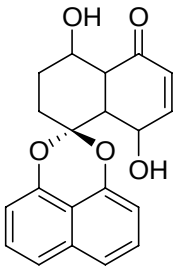


3.7

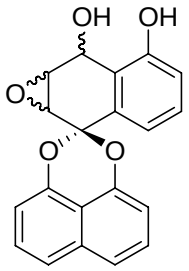


3.8

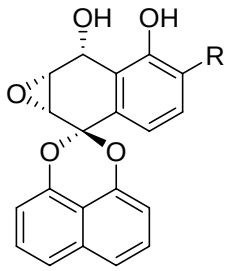
Considering its simple monomeric napthol building blocks, this class of natural products shows a huge diversity. Extensive reduction or oxidation (including epoxidation in the diepoxins) of the naphthalene cores is common, and other chemical modifications such as methylation and halogenation have also been observed. They are examples of how proficient nature is in exploring chemical space with simple starting materials. In his 2003 review of the spirobisnaphthalenes, Krohn summarised more than 60 naturally-occurring analogues, all found since 1990, some examples of which are shown in **Figure 3.1**.[8] This demonstrates the rapid growth of this important class of natural products.



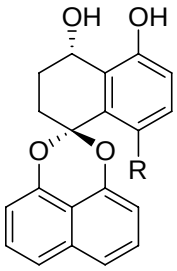
MK3018



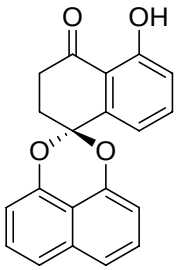
Bipendensin and Palmarumycin C_{11}



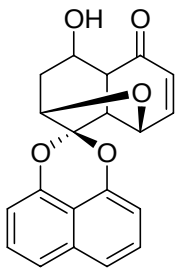
SCH 53823 (R=H)
SCH 53825 (R=Cl)



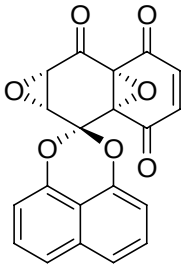
CJ-12,371 (R=H)
CJ-12,372 (R=OH)



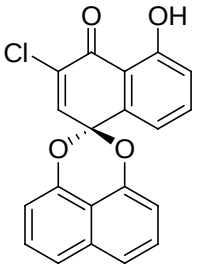
Palmarumycin CP_2
(Deoxypreussomerin B)



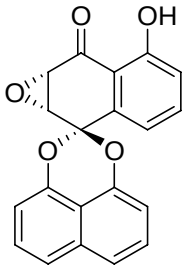
Palmarumycin CP_3



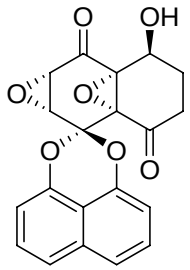
Palmarumycin C_9



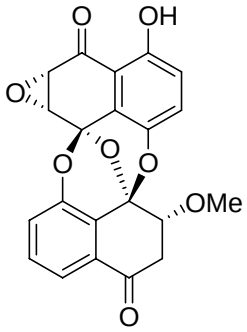
Palmarumycin C_1



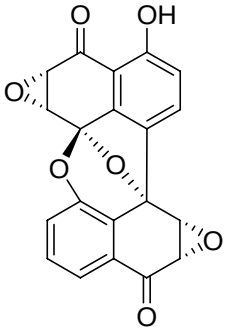
Palmarumycin C_2



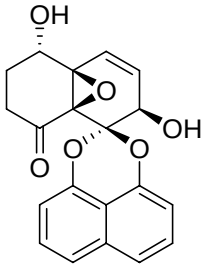
Diepoxin 1



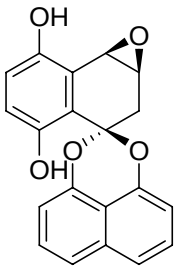
Preussomerin I



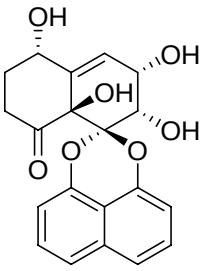
Spiroxin C



Cladospirone E



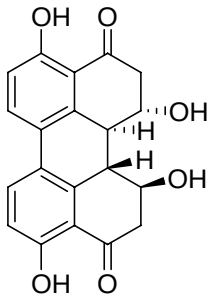
Cladospirone F



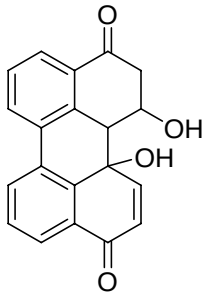
Cladospirone I

Figure 3.1 – Further examples of the spirobisnaphthalene class of natural products[2,6,7,9,10,11,12,13,14]

There are a number of related bisnaphthalene natural products with alternative naphthalene bridging motifs. Stemphytriol (**3.9**) and the phytotoxin alteichin (**3.10**) are fungal metabolites.[15,16] Sphaerolone (**3.11**) was produced when the normal spirobisnaphthalene biosynthetic pathway of the *Sphaeropsidales* sp. fungus was disrupted by inhibiting an enzyme involved in dihydroxynaphthalene biosynthesis (*vide infra*).[17] These compounds will not be discussed further, however, as they have a fundamentally different skeleton to the spirobisnaphthalenes.



3.9



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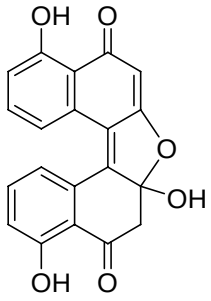
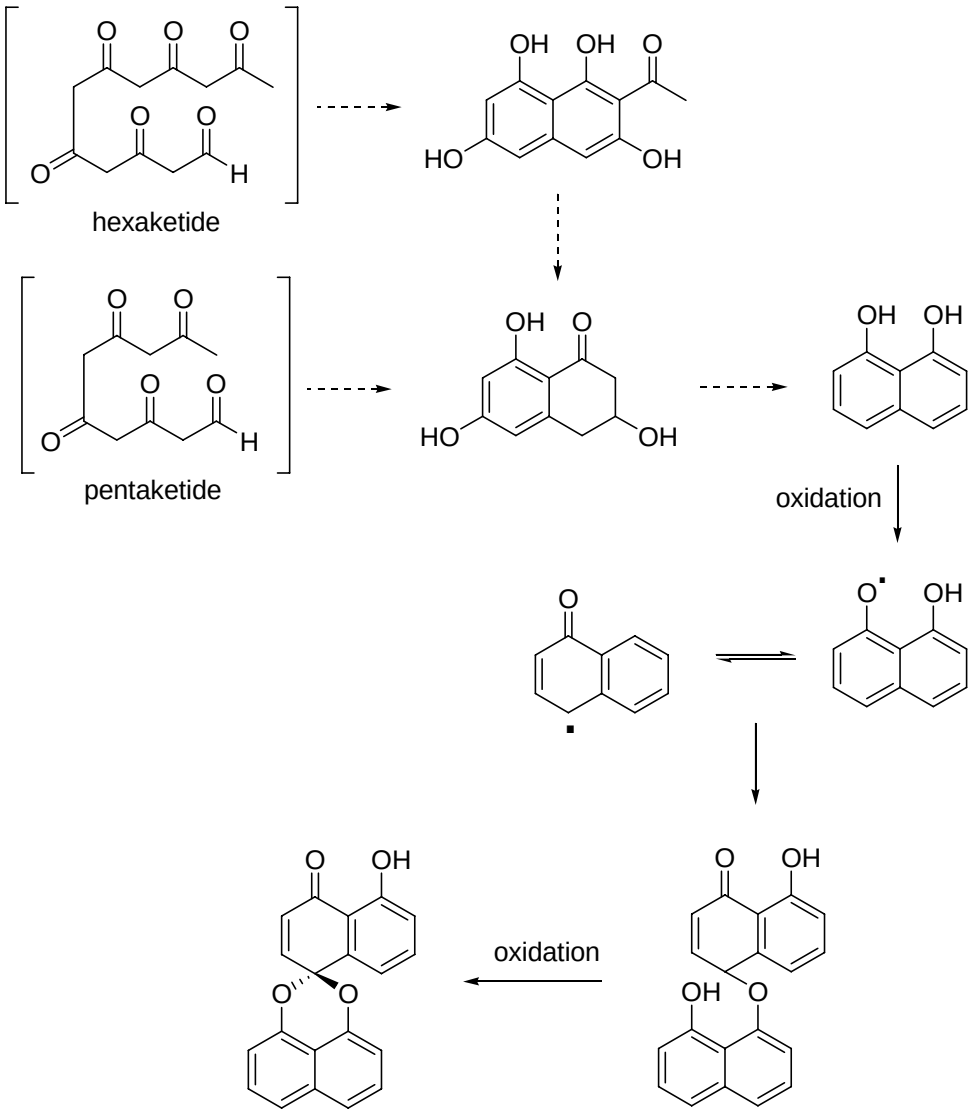


3.11

In addition to their interesting structures, the spirobisnaphthalene class also display diverse biological activities. Antibacterial and antifungal properties are common, for example, amongst the palmarumycins, the diepoxins and the preussomerins. Herbicidal activity has been noted in cladospirone bisepoxide[18] and some spirobisnaphthalenes have demonstrated antitumour poperties, such as the spiroxins. The preussomerins have been shown to selectively inhibit farnesyl-protein transferase,[13] an enzyme involved in post-translational modification of many proteins involved in intracellular signalling. The oncologically mutated form of one of these proteins is found in over 30% of human cancers. Selective inhibitors of this enzyme are therefore being developed as potential antitumour agents.

The biosynthetic origin of the spirobisnaphthalenes has been speculated upon and studied by a number of groups. Krohn *et al*. postulated the intermediacy of 1,8-dihydroxynaphthalene, DHN, a fungal metabolite derived from a penta- or hexaketide (**Scheme 3.1**).[9] DHN is one of the precursors used by fungi to produce melanins, which

are highly coloured polymers.[19] Melanins are found in several animal kingdoms, and whilst not essential for survival, they have a range of functions which increase organism fitness. For example, melanin's ability to absorb UV radiation reduces environmental cell damage, and is useful in camouflage. The ink secreted by squid, as a defence mechanism, is a fine suspension of melanin granules. Melanin also increases resistance to hydrolytic enzymes in fungi, and melanin precursors or derivatives (including DHN) often have antibiotic activity or phytotoxic activity (eg alteichin, **3.10**).



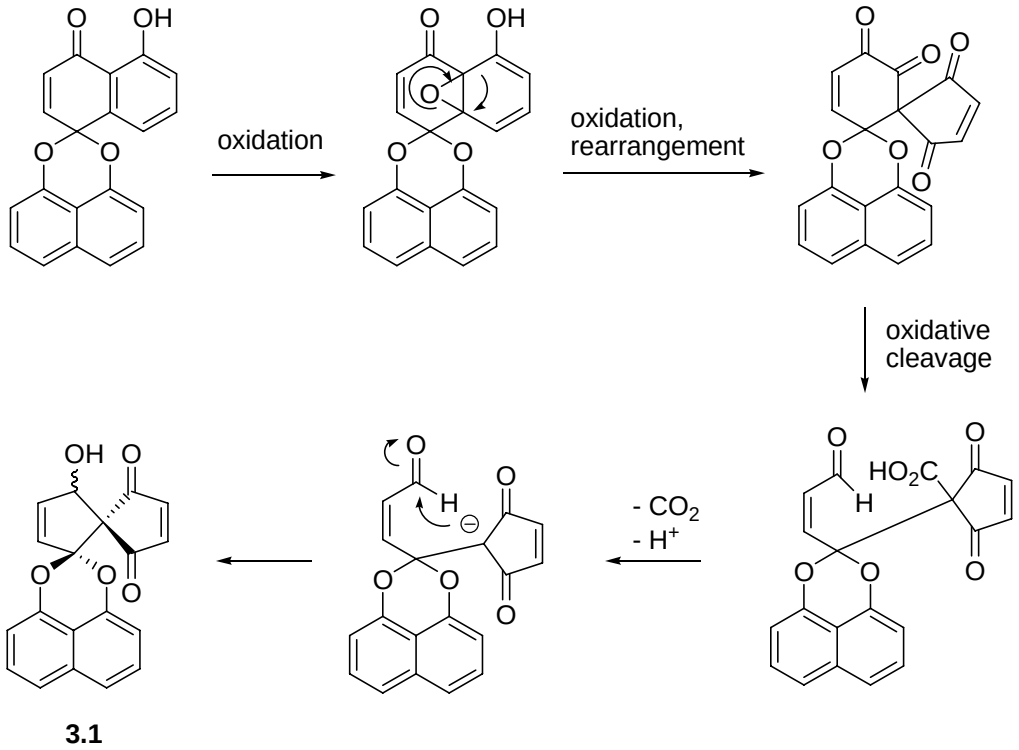
Scheme 3.1 – Krohn and co-workers' proposed biosynthetic origin for Palmarumycin CP_1

Krohn *et al*. proposed that fungi also use DHN as a precursor in the elaborate and specialised biosynthesis of the spirobisnaphthalene class. Phenol oxidation and coupling provides the core skeleton, which on further enzymatic processing, allows biosynthesis of this vast array of natural products. The same group studied the plausibility of the key oxidative spiroketal formation by forming palmarumycin CP_1 by oxidation of a naturally occurring biosynthetic precursor (discussed further in **Section 3.1.2**, **Scheme 3.3**).[20]

Several experimental studies on the biosynthesis of spirobisnaphthalene natural products were carried out by Bode *et al*.. The polyketide nature of these natural products was confirmed using labelling studies.[21] In addition, the intermediacy of 1,8-dihydroxynaphthalene (DHN) was confirmed by simultaneous inhibition of cladospirone bisepoxide and DHN melanin biosynthesis using a small molecule inhibitor.[17] Interesting new natural products were produced by the organism during the course of this experiment (including sphaerolone, **3.11**), derived from oxidative coupling of DHN precursors. Finally, variation of culture conditions also yielded insight into the regulation of biosynthetic pathways.[14] For example, oxygen levels during fermentation of a spirobisnaphthalene producing fungus affected the production of various oxygenated or reduced spirobisnaphthalenes.

The *spiro*-mamakone series, whilst clearly structurally and biosynthetically related, have a markedly different carbon skeleton to the rest of the spirobisnaphthalene class. One of the naphthalene units has one carbon extracted, and an interesting nine-membered, *spiro*-bicyclic unit results. This represents an interesting tangent on the biosynthetic pathway of spirobisnaphthalene natural products, and is a further example of the fascinating ways nature explores diverse chemical space using modification of simple precursors. It has been shown by Drs van der Sar and Mitova in the University of Canterbury natural products group that the biosynthetic origin of this skeletal modification involves the oxidation and rearrangement of an epoxide intermediate, with loss of CO_2 (**Scheme 3.2**).[22] Interestingly, *spiro*-mamakone A, **3.1**, is a rare example of a racemic natural product, which can be accounted for by the proposed biosynthetic pathway, which was formulated on the basis of unnatural isotope incorporation studies. The *spiro*-bicyclic

moiety of the *spiro*-mamakones is characterised by extensive oxidation, and the degree of oxidation varies across the series.

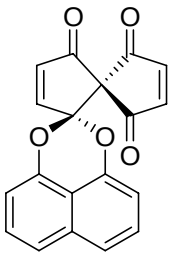


3.1

Scheme 3.2 – Proposed biosynthesis of *spiro*-mamakone A

The *spiro*-mamakones display some potent bioactivities. *spiro*-Mamakone A (**3.1**) displayed potent cytotoxicity (IC_{50} of 0.33 μM in the murine leukaemia cell line P388 assay) and showed potent antimicrobial activity against *Bacillus subtilis*, *Trichophyton mentagrophytes* and *Cladosporium resinae*. *spiro*-Mamakone B (**3.2**) and C (**3.3**) showed cytotoxicity in the P388 assay (1.39 μM and 0.95 μM respectively) and moderate antimicrobial activity against *B. subtilis* and *T. mentagrophytes*. *spiro*-Mamakone D (**3.4**) showed a cytotoxicity of 3.76 μM, but was not antimicrobial. No biological data for *spiro*-mamakone E (**3.5**) were obtained.

During studies of the *spiro*-mamakones, a semi-synthetic analogue was prepared by Dr van der Sar, *spiro*-mamakone F, **3.12**. This was prepared by oxidation of the racemic alcohol of *spiro*-mamakone A and also displayed cytotoxicity (1.13 μM) and moderate antimicrobial activity (*B.subtilis*, *T. mentagrophytes,* and *C. resinae*).

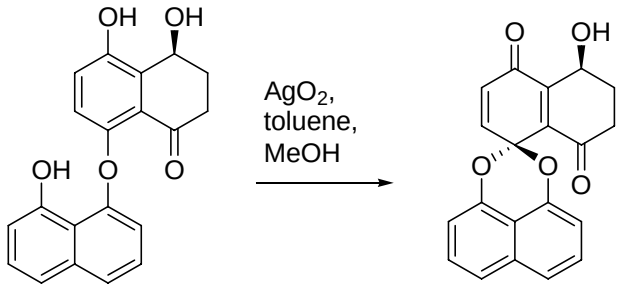


3.12

The compact but highly functionalised nature of the *spiro*-mamakones and their biological activities make them attractive and challenging synthetic targets.

3.1.2 Spirobisnaphthalene synthesis – a literature review

Whilst the isolation of the spirobisnaphthalene class of natural products began in the early 1990s and a massive array of natural compounds rapidly amassed in the literature, it was not until 1997 that the first tentative studies of their synthesis appeared in the literature. In that year, a model study for the synthesis of diepoxin σ was reported by Wipf and Jung,[23] and a semi-synthetic preparation of a palmarumycin analogue (**3.14**) from a naturally occurring, "open-chain" analogue, **3.13**, was described by Krohn *et al*. (**Scheme 3.3**).[20] The latter study supports the proposed biosynthetic route to the spirobisnaphthalenes, involving oxidative cyclisation of phenolic bisnaphthyl ethers.



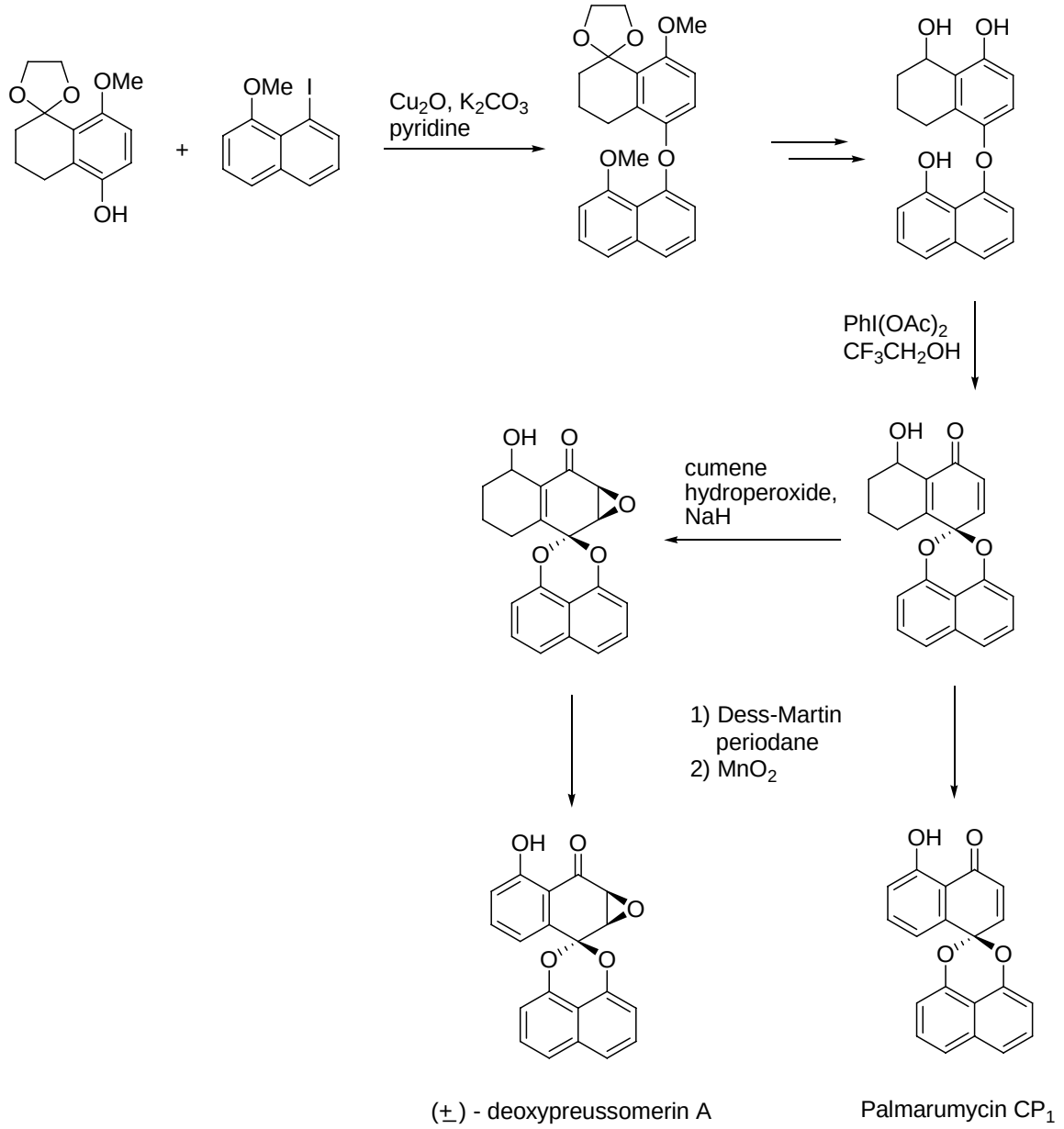
3.13 **3.14**

Scheme 3.3 – Semi-synthesis of a palmarumycin analogue

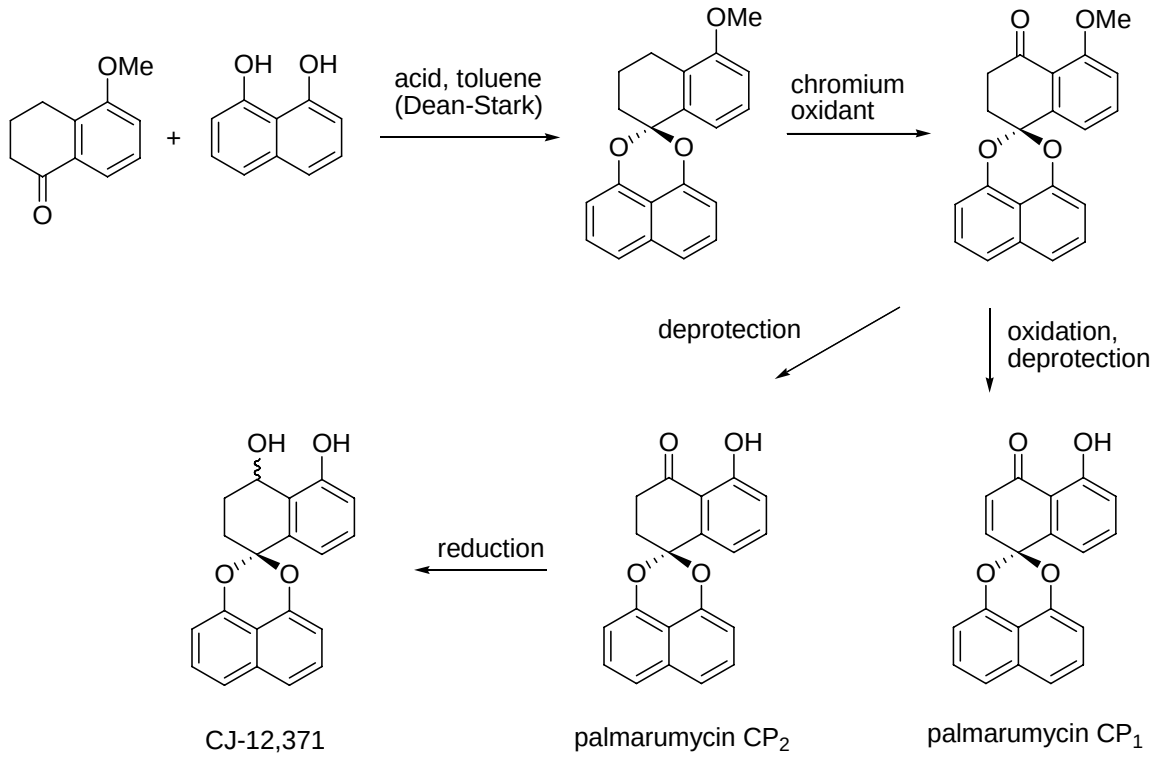
After these two initial reports, the floodgates were opened, however, and in the last ten years, several groups have reported significant progress in the synthesis of the spirobisnaphthalene class, with many natural products having succumbed to total synthesis. These synthetic efforts have covered several different subsets of the spirobisnaphthalenes including the palmarumycins, preussomerins, diepoxins and the spiroxins. Stereoselective syntheses of chiral spirobisnaphthalenes have recently been reported. This burgeoning field has also helped shed light on biosynthetic aspects of the spirobisnaphthalenes. A number of different strategies have been developed to access the crucial bisnaphthyl spiroketal moiety, using both "biomimetic" and non-biomimetic methods.

In early 1998, the three main contenders in this field, Wipf, Taylor and Barrett, and co-workers, all published their first syntheses of spirobisnaphthalene natural products. Wipf and Jung reported the synthesis of palmarumycin CP_1 and (±)-deoxypreussomerin.[24] This involved a "biomimetic" strategy for spiroketal formation, by formation of a phenolic bisnaphthyl ether employing the Ullman coupling of a phenol and an iodo- derivative. The ether then underwent an oxidative spirocyclisation with a proximal phenol to form the spiroketal (**Scheme 3.4**). This work was extended in a later publication, to include an array of analogues where Mitsunobu conditions were used to form ethers from the palmarumycin phenol.[25]

Barrett and Taylor, and co-workers, published very similar strategies to palmarumycin CP_1, CP_2 and CJ-12,371 (**Scheme 3.5**).[26,27] The precise reagents used were different, however, the overall strategy was identical. Barrett's synthesis included an asymmetric reduction to give CJ-12,371 (*S*) in good enantiomeric excess by use of chiral reducing agent (+)-*B*-chlorodiisopinocamphenylborane.

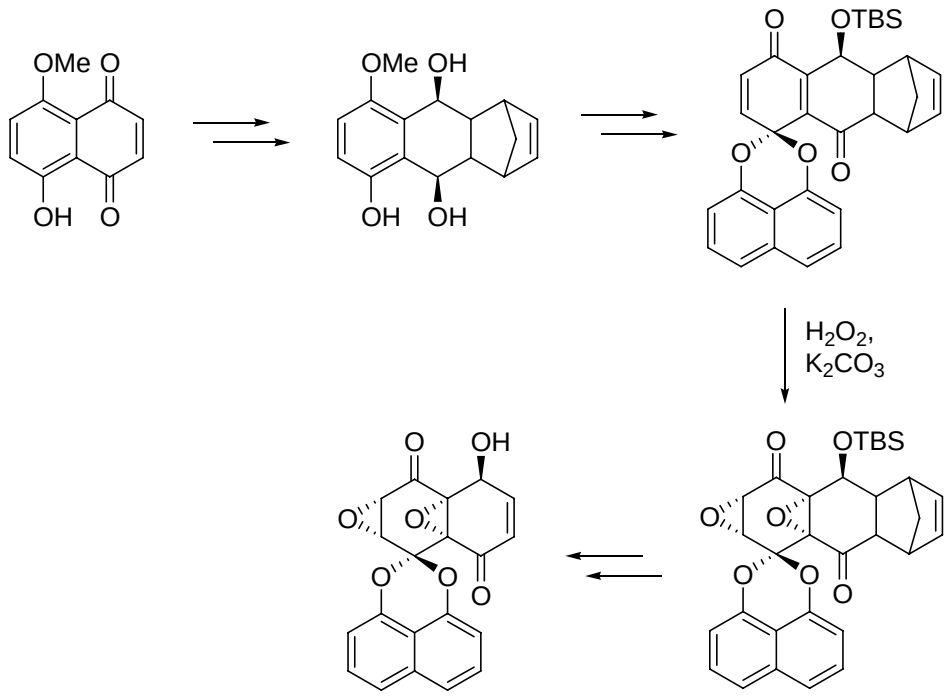


Scheme 3.4 – Wipf and Jung's synthesis of spirobisnaphthalene natural products

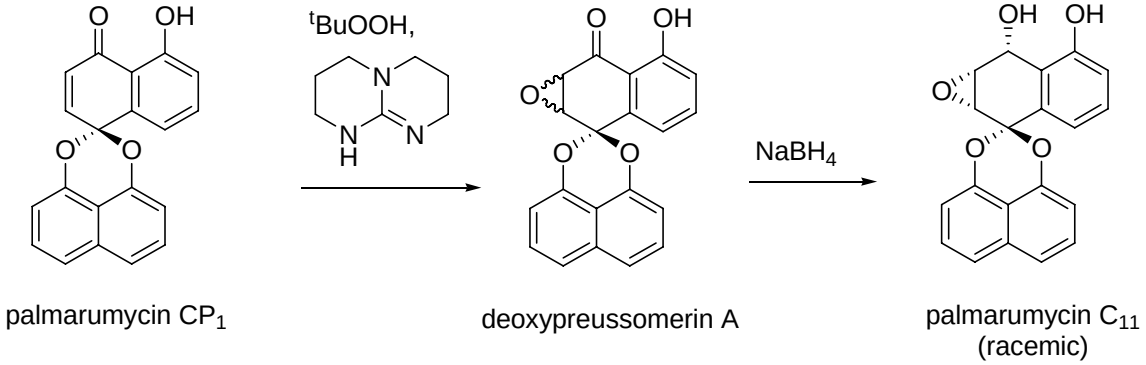


Scheme 3.5 – Barrett and Taylor's synthetic route to spirobisnaphthalene natural products

In 1999, Wipf and Taylor both extended their strategies to new spirobisnaphthalene natural products. Wipf and Jung successfully synthesised (±)-diepoxin σ, with good levels of diastereoisomeric excess during the double epoxide formation (**Scheme 3.6**).[28] Taylor *et al*. synthesised deoxypreussomerin A by epoxidation of palmarumycin CP_1, and subsequent reduction of deoxypreussomerin A yielded palmarumycin CP_{11} (**Scheme 3.7**).[29]

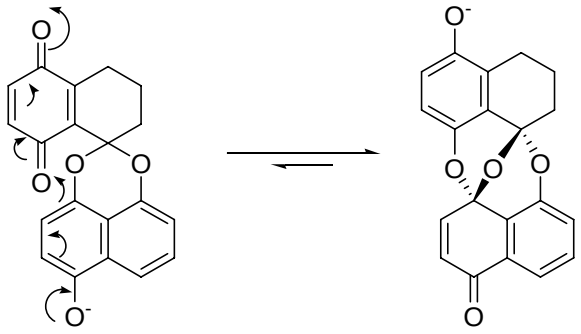


Scheme 3.6 – Wipf and Jung's synthesis of (±)-diepoxin σ



Scheme 3.7 – Further spirobisnaphthalenes from the group of Taylor

Also in 1999, Chi and Heathcock reported the first synthesis of members of the preussomerin group (G and I), where the naphthalene units are joined by three oxygen bridges.[30] The formation of the key bis-spiroketal moiety was carried out by a "ring-chain tautomerisation" in what is believed to be a biomimetic mechanism (**Scheme 3.8**). The resonance energy gained during the formation of two isolated aromatic systems of **3.16** from one naphthalene in **3.15** drives this reaction. Further synthetic manipulation of **3.16** afforded preussomerins G and I.

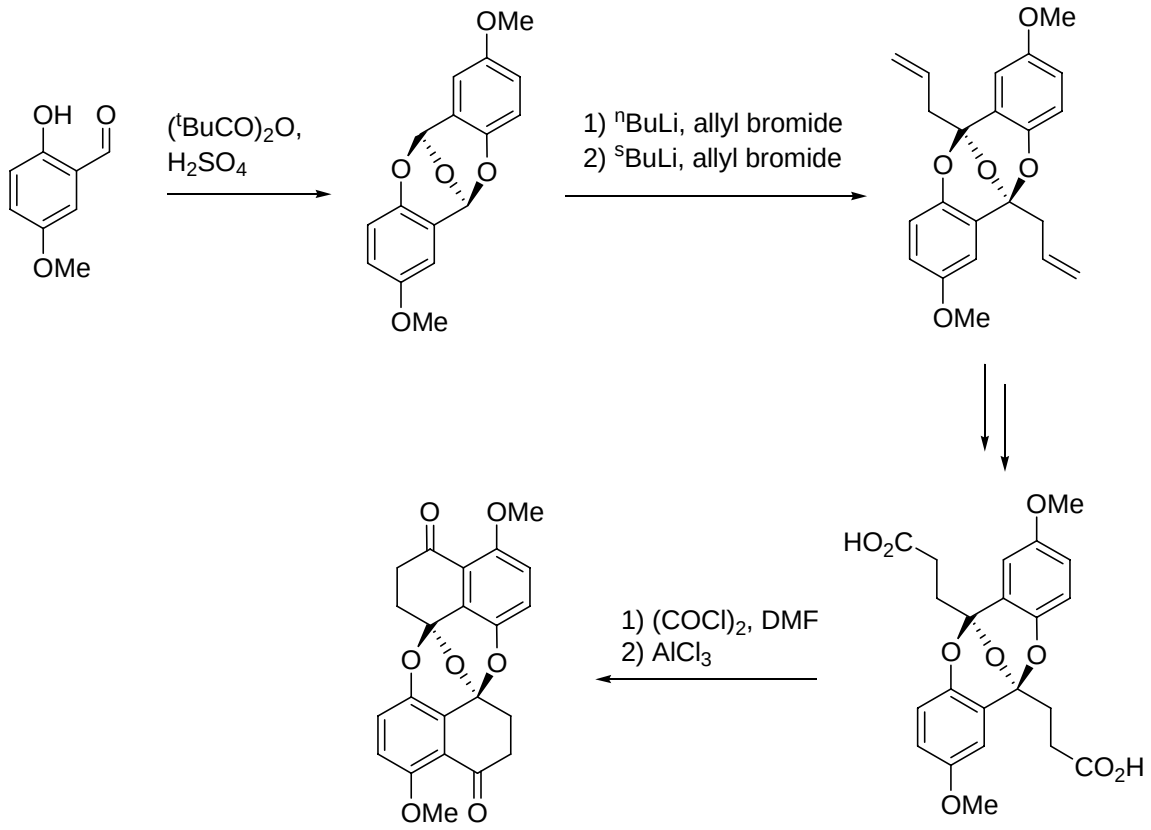


3.15 **3.16**

Scheme 3.8 – Biomimetic bis-spiroketal formation demonstrated by Chi and Heathcock

Taylor and Wipf reported further progress in this field in 2000, with Wipf and Jung reporting the formal asymmetric synthesis of (+)-diepoxin σ and Taylor *et al*. describing a new, non-biomimetic approach to the bis-spironaphthyl core. Wipf's asymmetric synthesis of (+)-diepoxin σ relied on the asymmetric introduction (using a chiral boron complex) of a cyclopentadiene Diels-Alder derived protected double bond.[31] This bulky protecting group served as a stereochemical anchor which could be removed at 250-260ºC (refluxing phenyl ether), conditions under which the compound is stable.

Taylor *et al*. synthesised a preussomerin analogue using an unusual aryl acetal anion strategy (**Scheme 3.9**). [32] They synthesised the bis-spiroketal core by dimerisation of salicyl aldehydes. They then showed that these moieties could be successively deprotonated and alkylated, and after oxidation and a Freidel-Crafts cyclisation afforded the preussomerin skeleton. This group would later go on to demonstrate the utility of this approach to preussomerins by synthesising (±)-preussomerins F, K and L.[33,34]



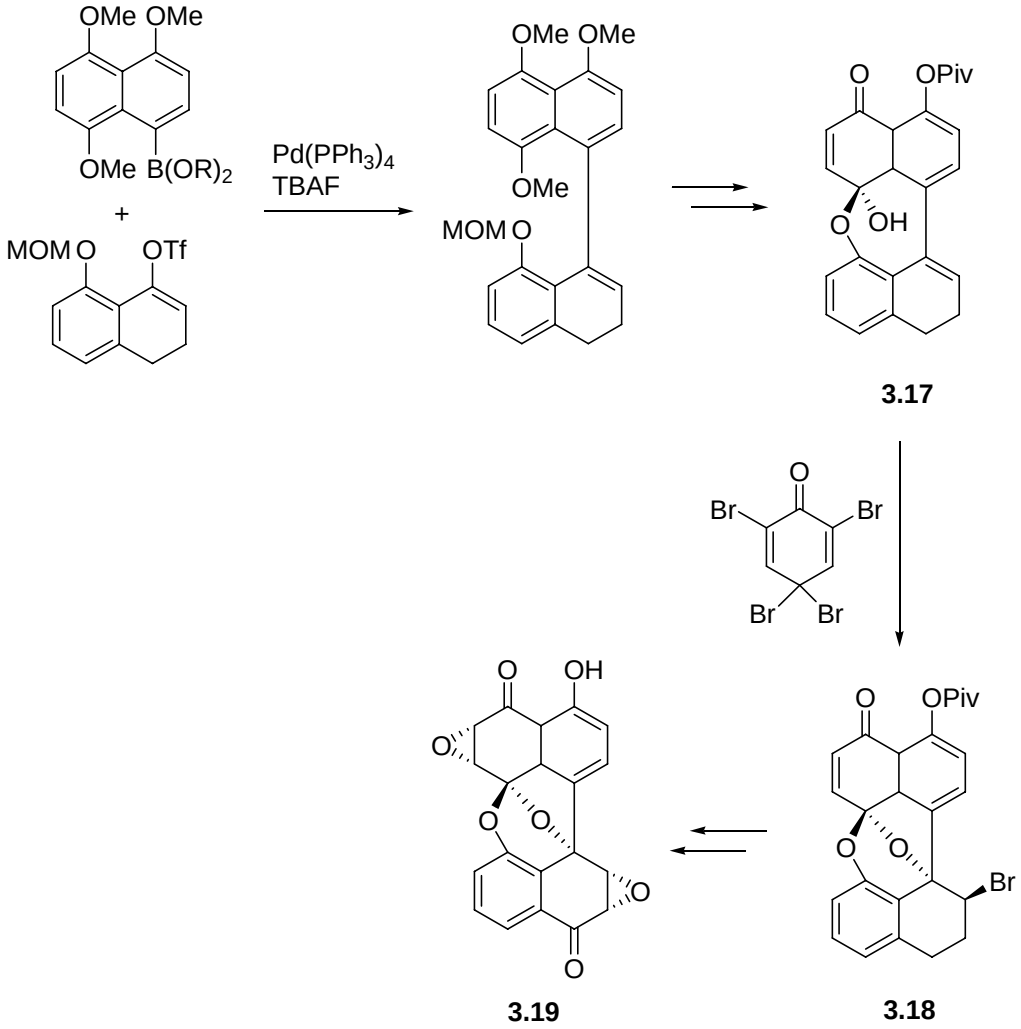
Scheme 3.9 – Novel approach to the preussomerin skeleton

In 2000, Coutts *et al*. published a novel approach to the palmarumycin skeleton in a manner similar to the biomimetic method, employed by Wipf and Jung.[35] The crucial difference was the replacement of the Ullman coupling with a nucleophilic aromatic substitution on an activated aryl fluoride by a phenol under basic conditions. They used this route to make a number of palmarumycin analogues.

In 2002, Barrett *et al*. described a unified route to the palmarumycin and preussomerin core structures, employing a strategy similar to Chi and Heathcock.[36] The authors also described the stereoselective synthesis of (-)-preussomerin G, with stereoselectivity induced by use of a chiral phase transfer reagent during epoxidation.

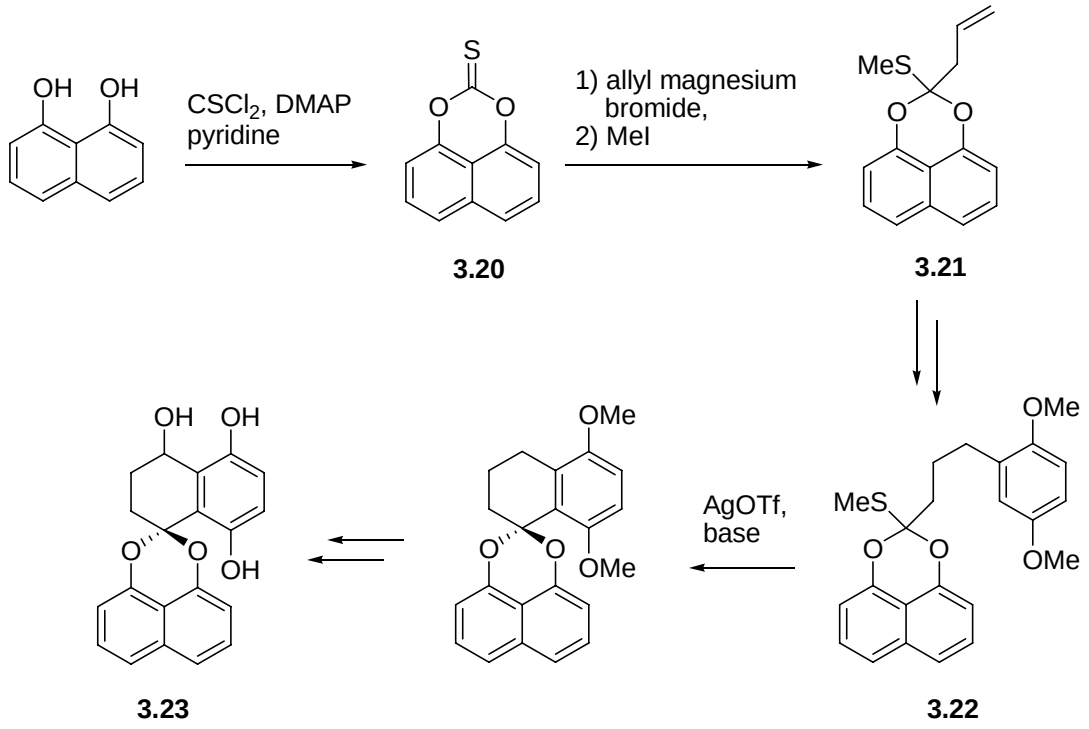
2003 saw the first synthesis of the spiroxin subset of the spirobisnaphthalene class, with the synthesis of (±)-spiroxin by Miyashita and co-workers.[37] In these natural products, the two naphthalene groups are bridged by both a spiroketal, and a carbon-carbon bond.

The authors first tackled the carbon-carbon bond, via a Suzuki-Miyaura cross-coupling reaction (**Scheme 3.10**). Deprotection of a phenol formed a protected hemiacetal, **3.17**, which, after deprotection, formed the spiroketal, **3.18**, by intramolecular addition to a double bond in the presence of a brominating reagent. Subsequent synthetic manipulation yielded (±)-spiroxin C (**3.19**).



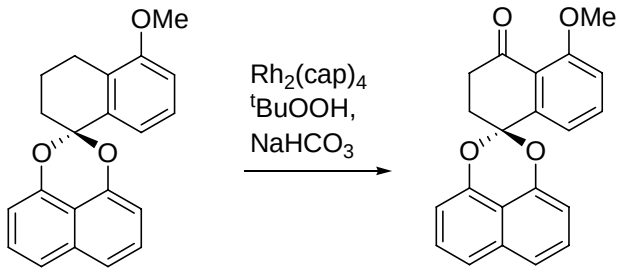
Scheme 3.10 – Miyashita and co-workers' synthesis of (±)-spiroxin C (**3.19**)

The report of Inoue and co-workers presented a novel approach to the spiroketal moiety, which was employed in these authors' synthesis of CJ-12,372 (**Scheme 3.11**). They generated thiocarbonate, **3.20**, which underwent nucleophilic addition and was trapped as the monothioorthoester, **3.21**. A Suzuki-Miyaura coupling yielded the precursor, **3.22**, for cationic cyclisation which provided the basic spirobisnaphthalene skeleton. After further synthetic manipulation, (±)-CJ-12,372 (**3.23**) was successfully synthesised. This unusual approach to the spirobisnaphthalene skeleton has not been employed by any other groups to date.



Scheme 3.11 – Alternative approach to spiroketal moiety and synthesis of (±)-CJ-12,372, **3.23**

The most recent report of a spirobisnaphthalene synthesis was the application of a new benzylic oxidation rhodium catalyst in the conversion of a late stage intermediate to palmarumycin CP_2 (**3.24**, **Scheme 3.12**) described by Catino and co-workers.[38]



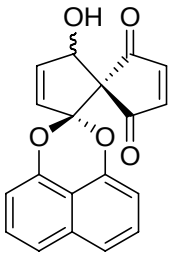
3.24

Scheme 3.12 – Palmarumycin CP_2, **3.24**, synthesis using new rhodium benzylic oxidation catalyst

With such a structurally diverse class of natural products as the spirobisnaphthalenes, generalisation of their synthesis is not trivial. However, a common thread through most synthetic routes to spirobisnaphthalenes is the early introduction of the crucial spiroketal (or bis-spiroketal) moiety, followed by varying degrees of subsequent manipulation. A wide variety of reaction conditions have been employed on the spiroketal bisnaphthyl cores, with oxidative and reductive processes among the most heavily utilised. The ability to introduce the spiroketal function so early in these syntheses relies on the stability that it displays to a wide range of conditions. Even under acidic conditions, the ketal has been shown to be surprisingly stable; surviving refluxing methanol and aqueous HCl mixtures, and boiling in acetic acid.[9]

3.1.3 Synthetic strategies to spiro-*mamakone A*

Due to both the interesting structural features of the *spiro*-mamakones and their biological activity, their synthesis is of interest. The successful synthesis of related spirobisnaphthalenes suggests that they are a realistic, albeit challenging synthetic target. *spiro*-Mamakone A, **3.1**, was the principal synthetic target, however, the structural features of the other *spiro*-mamakones were borne in mind throughout these synthetic explorations in the hope of generating a unified route to the series.



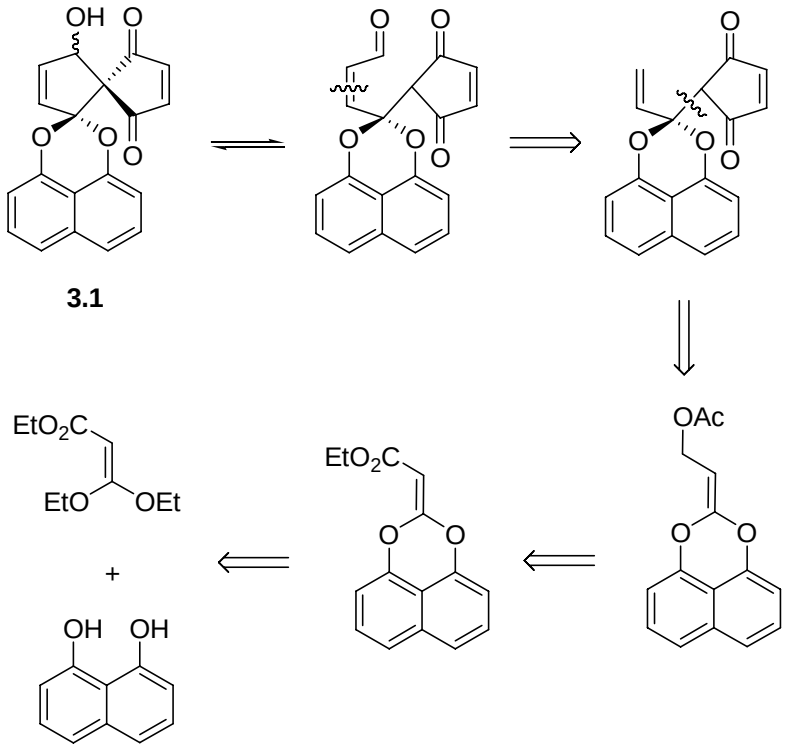
3.1

When designing a synthetic route to *spiro*-mamakone A, the main structural difference to the other spirobisnapthalenes is the nine carbon *spiro*-bicyclic moiety which replaces the ten carbon naphthalenoid moiety. This would require a different strategy to this half of the molecule, however, the overall nature of *spiro*-mamakone A is otherwise very similar to the other spirobisnaphthalenes. It was therefore assumed that the synthetic considerations appearing in the literature for the spirobisnaphthalenes (discussed above, **Section 3.1.2**) could be broadly applied to *spiro*-mamakone A. For example, it seemed reasonable to expect the spiroketal functionality to be of a similar stability in both classes, allowing its formation at an early stage of the synthesis from an appropriate pair of nine and ten-membered bicyclic structures. With a basic *spiro*-mamakone skeleton thus in place, it was anticipated that manipulation of the oxidation state of the heavily functionalised northern hemisphere would be possible.

Three basic methods for generating the key spirobisnaphthalene spiroketal have been published, as discussed previously (**Section 3.1.2**). First, the "biomimetic" Ullman coupling method, as employed by Wipf *et al.* (**Scheme 3.4**), where an ether is first formed between two aryl systems (an aryl iodide and phenol) using copper mediated nucleophilic aromatic substitution. A proximal phenol is then involved in an oxidative ring-closing ketalisation reaction. Coutts *et al.* described a similar approach using an aryl fluoride. This approach is, however, not easily applicable to *spiro*-mamakone A. The reaction is applicable to those spirobisnapthalenes where the final product is, or an intermediate can be derived from, two aromatic systems. This can be envisioned for all spirobisnaphthalenes with the exception of the *spiro*-mamakones, since the northern hemisphere involves two 5-membered rings connected by a *spiro*- centre. Whilst five-

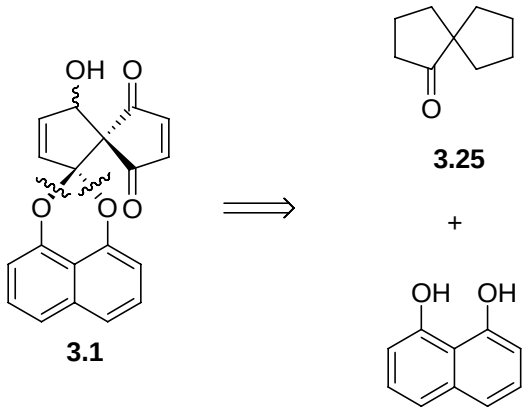
membered aromatic systems such as cylcopentadiene anions can react in Ullman type reactions[39] a *spiro*-disubstituted cyclopentadiene could not be deprotonated and therefore can not be aromatised.

The second method is the non-biomimetic approach of Inoue *et al.* (**Scheme 3.11**). Here DHN is functionalised to generate an activated 'ketal' with an electrophilic centre which can then undergo nucleophilic addition. After further functionalisation, ring-closing yields the final spirobisnaphthalene skeleton. This sort of approach is more amenable to the synthesis of *spiro*-mamakone A and one route to *spiro*-mamakone A which was briefly examined (retrosynthesis described in **Scheme 3.13**) is related to this one, and is described further later (**Section 3.2.1**).



Scheme 3.13 – Retrosynthetic analysis of *spiro*-mamakone A, **3.1**, using an approach related to that of Inoue and co-workers

The final, non-biomimetic method for spiroketal formation used by Taylor and Barrett (eg **Scheme 3.5**) involves classic ketal formation using an acid catalysed dehydration reaction between a diol and a ketone. This approach would be expected to be appropriate for the synthesis of *spiro*-mamakone A (retrosynthetic analysis shown in **Scheme 3.14**). The use of DHN as diol that would be required for synthesis of *spiro*-mamakone A was successfully employed for spirobisnaphthalene synthesis by these authors. It is fair to assume that an appropriate nine-membered ketone might react similarly to the tetralone employed by the authors, to form the *spiro*-mamakone skeleton. This approach therefore requires the design of an appropriate ketone for ketalisation with DHN.

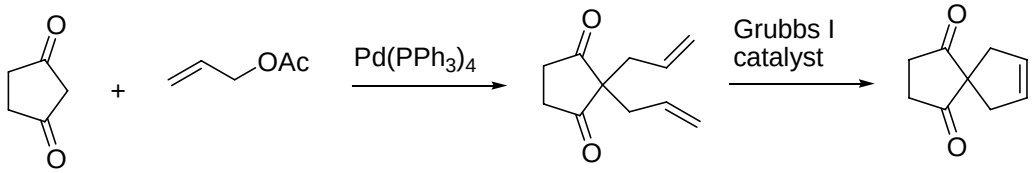


Scheme 3.14 – Gross retrosynthetic analysis of *spiro*-mamakone A related to approach of Taylor and Barrett and co-workers

Since there should be an opportunity to functionalise the northern hemisphere after construction of the spiroketal, the exact nature of ketone **3.25** can be varied for ease of access. Certain restrictions on ketone **3.25** do exist, however. For example, heavily conjugated ketones tend to form ketals only reluctantly, so an unconjugated ketone would be preferred. The presence of appropriate functionality in both rings of ketone **3.25** is required to allow manipulation of the *spiro*-mamakone skeleton to give the final target compound.

A number of different ketones were considered for this purpose, however, a search of the literature brought to light a very promising ketone, **3.27**, whose two step synthesis was reported in high yields (**Scheme 3.15**).[40,41] This nine-carbon bicyclic ketone appeared to

be an excellent starting point for an attempt to synthesise the *spiro*-mamakone carbon-skeleton and offered differential functionalities in each ring for further functionalisation.



3.26 **3.27**

Scheme 3.15 – Kotha and co-workers synthesis of spirobicyclic **3.27**

Ketone **3.27** contains two non-conjugated ketones which would be expected to form a spiroketal readily. The possibility of forming two ketals from **3.27** must be considered, however steric hindrance may impede a second ketal forming. Differential functionality on each half of the spirobicyclic **3.27** should allow selective manipulation of the northern hemisphere after spiroketalisation. This would be of benefit since *spiro*-mamakone A is asymmetrically oxidised and differentiation of these two rings would be necessary. Finally, the synthesis of ketone **3.27** is already known, is succinct and proceeds in good yields. It does require the use of the moderately expensive Grubbs' catalyst, however, as an efficient and reliable catalyst, this does not detract significantly from the approach.

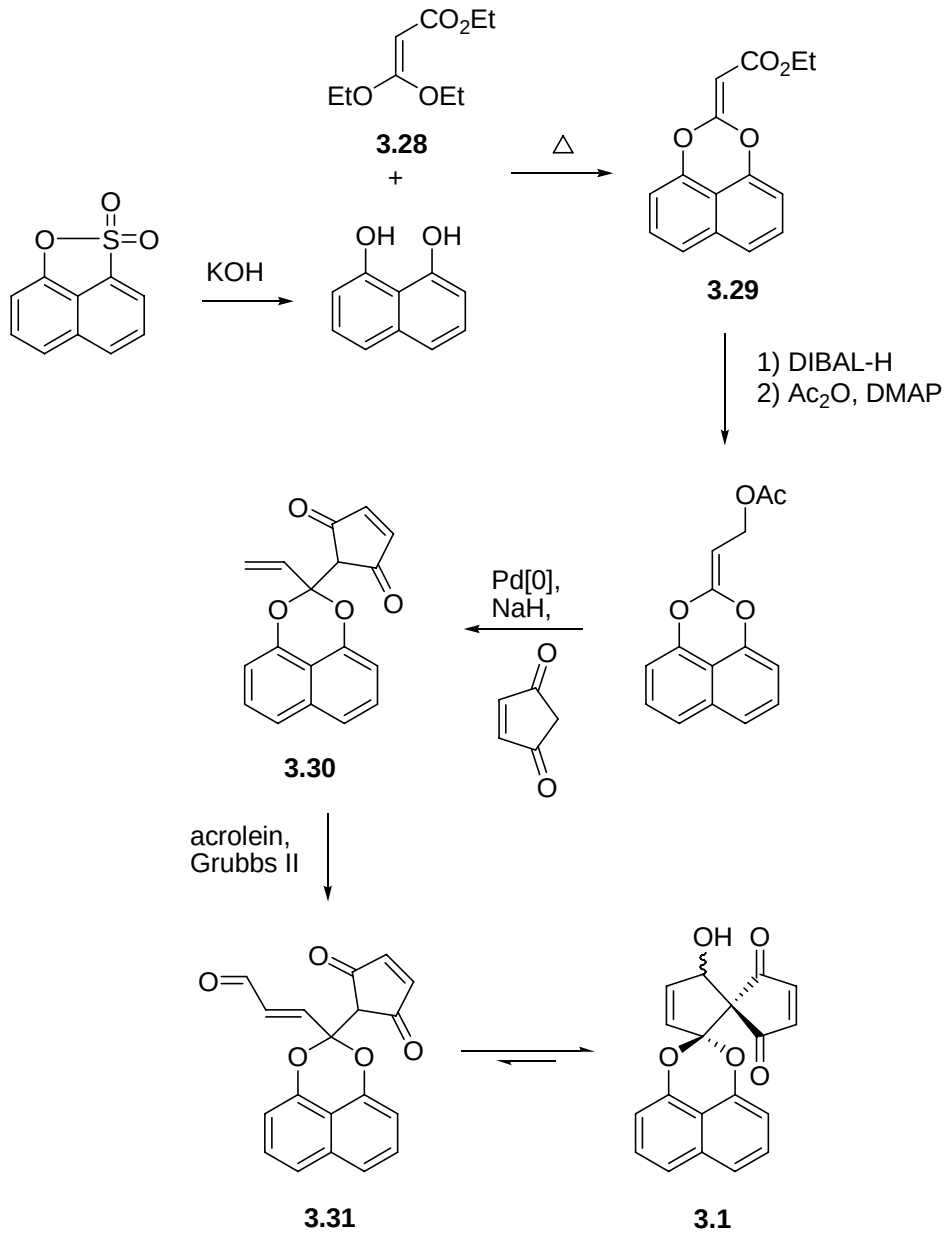
The high degree of functionality in so small a molecule as *spiro*-mamakone A gives the synthetic chemist a wide range of possibilities in the design of a synthetic strategy. Whilst other approaches were considered, the examples given above represent those approaches which seemed most promising. The first of these methods was not pursued extensively after falling at an early hurdle. Though further investigation may have allowed this hurdle to be cleared, it became clear that the other route (the ketalisation of ketone **3.27**) showed more promise. The "unsuccessful" route will be discussed initially (**Section 3.2.1**) with a more extensive discussion of the ultimately more successful route following (**Sections 3.2.2** to **3.2.10**).

3.2 Synthetic investigations

3.2.1 Investigation of Inoue-based approach

The first synthetic approach to *spiro*-mamakone A examined was that which was broadly based on the method of Inoue *et al.* (*vide supra*, **Scheme 3.13**). This synthetic method was kindly proposed by Professor Martin Banwell of the Australian National University, Canberra. A more detailed description of the synthetic route envisioned is given in **Scheme 3.16**.

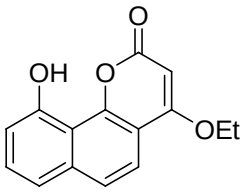
In this route, after formation of DHN using literature methods, equilibration with ethyl-3,3-ethoxyacrylate (**3.28**) would be expected to generate ketal **3.29**. Reduction of the ester to an alcohol and generation of a leaving group would generate an electrophilic centre which upon reaction with 4-cyclopentene-1,3-dione under basic conditions would rearrange to give terminal alkene **3.30**. After metathesis of the alkene with acrolein, aldehyde **3.31** would be anticipated to undergo a Knoevenagel isomerisation to give *spiro*-mamakone A. This final step can be considered to be biomimetic as it resembles the final intermediate proposed in the biosynthetic pathway to this natural product (**Scheme 3.2**).



Scheme 3.16 – Proposed synthetic route to *spiro*-mamakone A using an approach similar to Inoue and co-workers

The synthesis begins with the generation of 1,8-dihydroxynaphthalene. The method which has been reported to be most effective[29] is that by Erdmann, which involves fusion of 1,8-naphthosultone with potassium hydroxide.[42] This reaction was successfully repeated on scales of up to 6 g, with yields of around 70%.

The next step is an acetal exchange process between ketene acetal **3.28** and DHN. This reaction is an equilibration process, which would be expected to entropically favour the formation of desired **3.29**, as two molecules of ethanol would be generated. Acid is required to catalyse this process. Attempts were made to carry out this reaction, initially using DCM as solvent with catalytic quantities of pTSA. Carrying out the reaction overnight at either room temperature or reflux led only to recovery of starting materials. Carrying out the reaction in the absence of solvent at 45ºC did not improve the situation, although an impurity was observed. This impurity was the sole product of reaction at 85ºC, and could be purified and characterised as **3.32**, as described below, using ^{1}H (**Figure 3.2**), 2D and ^{13}C NMR spectroscopy and mass spectrometry.



3.32

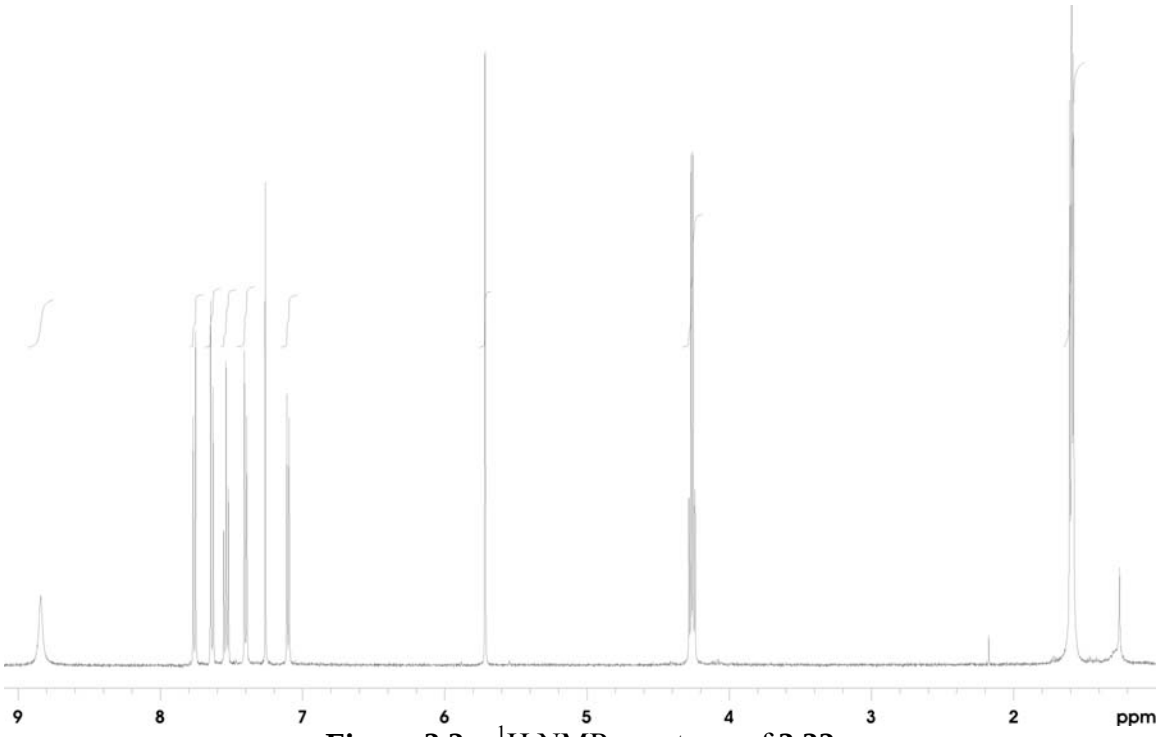


Figure 3.2 – ^{1}H NMR spectrum of **3.32**

The aromatic region of the ^{1}H NMR spectrum of the product did not show the typical pattern of the 1,2,3-trisubstituted rings of DHN. Instead one of the rings showed a 1,2,3,4-tetrasubstituted ring pattern. This suggested that substitution on the ring had occurred. The presence of only one up-field aromatic doublet suggested that the substituted proton was in the *ortho*-position relative to a phenol. The presence of one ethyl group suggested that only monosubstitution of the diethoxyacetal had occurred. Low resolution mass spectrometry (electron impact ionisation) showed that the pseudomolecular ion had a mass of 256 Dalton, corresponding to a molecular formula of $C_{15}H_{12}O_4$. This evidence allowed assignment of the product as **3.32**. One of the protons (δ_H 7.76) on the 1,2,3,4-tetrasubstituted ring showed a correlation in an HMBC NMR experiment to a carbonyl carbon at δ_C 167. The structure **3.32** makes this correlation a five-bond correlation, which is unusual, but in this heavily conjugated system, not impossible.

This is a surprising result and demonstrates that the phenolic oxygens are not efficient nucleophiles. The formation of **3.32** can be rationalised by an acid-catalysed attack on the electrophilic ketene acetal by the electron-rich phenol *ortho*-position, in a Friedel-Crafts alkylation-type reaction. After displacement of ethanol, the ester is ideally placed for an intramolecular lactonisation to form a six-membered ring. An alternative mechanism can be proposed which reverses these two steps. Thus, if the electrophilicity of the carbonyl were greater than that of the ketene acetal, initial attack by the phenol could occur at the ester. The electron-rich *ortho*-position would then be well placed to generate the six-membered ring of **3.32**. The latter of these two mechanisms is much less plausible, however, as the electrophilicity of the ketene acetal should be very high. Enones are good Michael acceptors and the additional electron withdrawing effects of the ethoxy substituents should make this a very electrophilic centre. Further evidence for the former mechanism was also found during the course of later experimentation (discussed further later, **Section 3.2.3**).

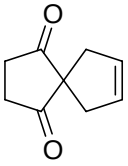
Whilst acetal exchange is generally catalysed by acid, the possibility of a base catalysed route was considered. The phenolic oxygen had been shown to be insufficiently

nucleophilic to initiate an acetal exchange, however a phenoxide would be significantly more nucleophilic. The use of catalytic sodium ethoxide would allow an equilibrium to set up which should favour the desired product. This reaction was attempted, however, no reaction was observed. Both starting materials were stable under the conditions (as observed by monitoring by ^{1}H NMR spectroscopy) except on refluxing the reaction (ethanol as solvent) when the ketene acetal decomposed.

With extensive experimentation, the problematic side-reactions and lack of reactivity may have been circumvented and the desired acetal exchange promoted, however, efforts were instead concentrated on other more promising approaches (**Section 3.2.2**).

3.2.2 Synthetic studies towards spiro-*mamakone A via nonadione 3.27*

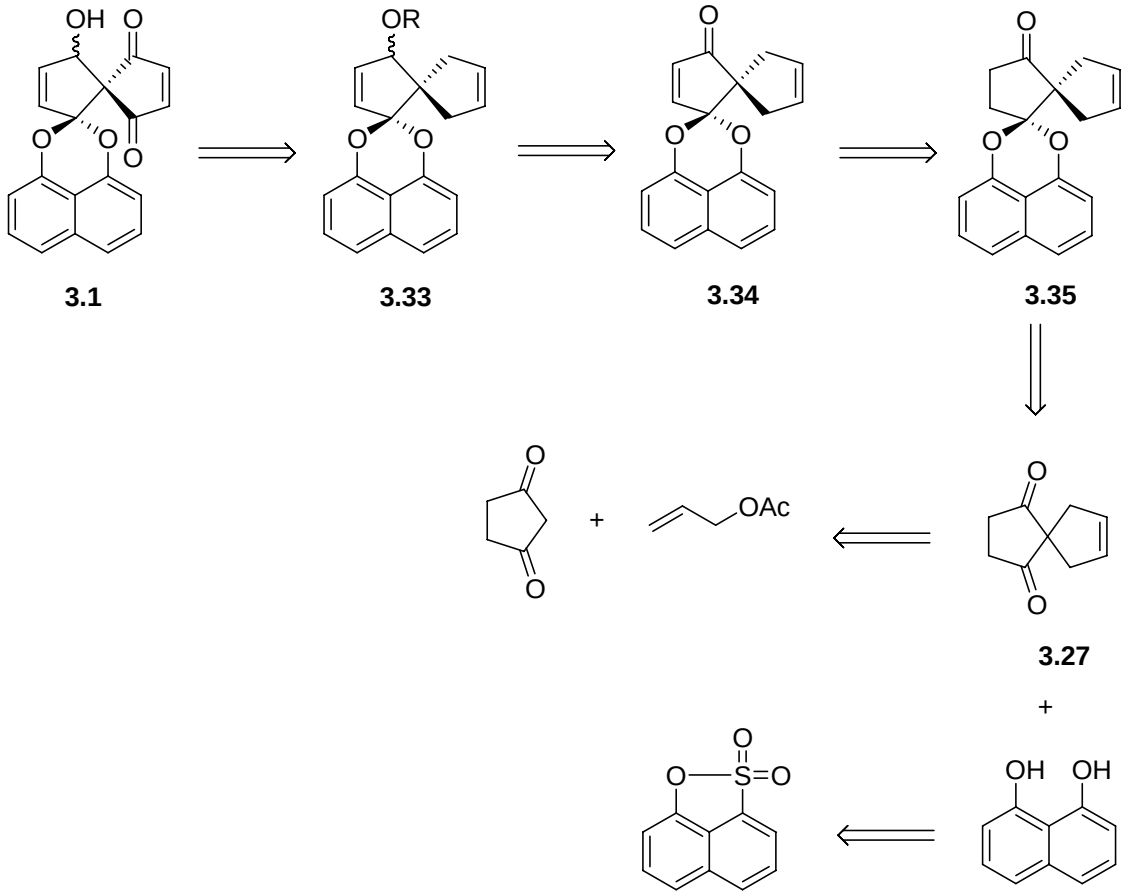
The most promising approach towards the synthesis of *spiro*-mamakone A was deemed to involve spiroketalisation of the known nonadione **3.27** with DHN and subsequent synthetic manipulation.



3.27

A detailed retrosynthetic analysis is shown in **Scheme 3.17**. The key spiroketalisation was envisioned to be carried out using methods described by Taylor *et al*. in their synthesis of spirobisnaphthalenes (*vide supra*). With the *spiro*-mamakone skeleton thus generated, the manipulation of the oxidation state of the northern hemisphere would have to be achieved. A variety of conditions are known which can achieve dehydrogenation of a ketone to yield an enone. Successful application of such a reaction to **3.35** would generate **3.34**. Selective 1,2-reduction of the enone **3.33** would introduce the allylic alcohol moiety required by the target *spiro*-mamakone A. The racemic nature of the natural product precludes the necessity to use asymmetric conditions for introduction of this moiety. Protection of this alcohol would be judicious before undertaking further

oxidative manipulations. Several methods have been reported to achieve allylic oxidation of alkenes first to enones and then to enediones. Successful double allylic oxidation of **3.33** to an enedione and cleavage of the allylic alcohol protecting group would then yield the final target *spiro*-mamakone A.


OH
O
OR
O
O
O

Having previously achieved the synthesis of DHN (*vide supra*), synthetic efforts towards *spiro*-mamakone A using this strategy therefore began with the preparation by literature methods of **3.27**. The procedure described by Kotha *et al.* was shown in **Scheme 3.15**.[40,41] Their synthesis of **3.26** is based on that described by Schwartz and Curran and calls for palladium-catalysed allylation of cyclopenta-1,3-dione using allyl acetate as the allyl source.[43] Cyclopenta-1,3-dione was available in-house, however allyl acetate was not available. A straightforward synthesis was therefore undertaken by following literature precedent and a moderate yield of 41% achieved.[44] The allylation reaction was then successfully carried out, however very low yields were encountered. This was found to be at least partly due to product volatility.

An alternative procedure for allylation of cyclopenta-1,3-dione to give **3.26** also exists in the literature.[45] This involved an alternative palladium(0) catalyst and allyl alcohol as the allyl source. The direct use of allyl alcohol would circumvent the need to synthesise allyl acetate (although it is also commercially available) and this procedure was also attempted. Improved yields were obtained by this method and it was the method employed throughout these synthetic studies. The volatility of **3.26** was found to diminish yields somewhat (around 85% isolated yield), however, this problem could be circumvented to give quantitative yields over this and the subsequent ring-closing step (*vide infra*). ^{1}H and ^{13}C NMR spectra of the product were in good agreement with literature values.

Kotha *et al.* employed ring-closing metathesis of **3.26** using Grubbs' first generation catalyst to yield ketone **3.27**. Due to the in-house availability of Grubbs' second generation, its known higher activity and improved air stability prompted the use of this catalyst as a substitute for the first generation version. The ring-closed product, **3.27**, was successfully generated in near quantitative yield using 4 mole% of catalyst. ^{1}H and ^{13}C NMR data and the melting point of the product were all in good agreement with literature values. The volatility and diminished yields obtained after chromatographic purification of **3.26** prompted the examination of carrying out the ring-closing metathesis on the crude **3.26**, obtained without chromatography. Pleasingly, it was found that the metathesis step was unaffected by palladium and other impurities contained in crude **3.26**. When crude

3.26 was used, all impurities could be removed after metathesis to yield pure **3.27** in almost quantitative yields over the two steps. This represents an improvement on the overall yield observed by Kotha *et al.* of 78%.

Whilst the product was pure by ^{1}H NMR spectroscopy, the colour of various batches of **3.27** varied from dark to light grey. This was likely due to trace amounts of ruthenium complexes remaining after purification from the Grubbs' catalysed ring-closing metathesis. It was found that **3.27** could be obtained as a pure white crystalline solid by recrystallisation (some batches required multiple recrystallisation steps) from diethyl ether. Sublimation of **3.27** was often observed on leaving the product over several weeks at room temperature. Large scale sublimation was therefore attempted in order to obtain high purity **3.27**. Whilst trace amounts of pure **3.27** could be obtained by sublimation at around 50ºC (either under reduced or atmospheric pressure), this was found not to be a good method for large scale purification. If higher temperatures were employed (around 80ºC) the process was somewhat faster, however ^{1}H NMR data (**Figure 3.3**) showed that significant quantities of a thermal degradation product were also collected.

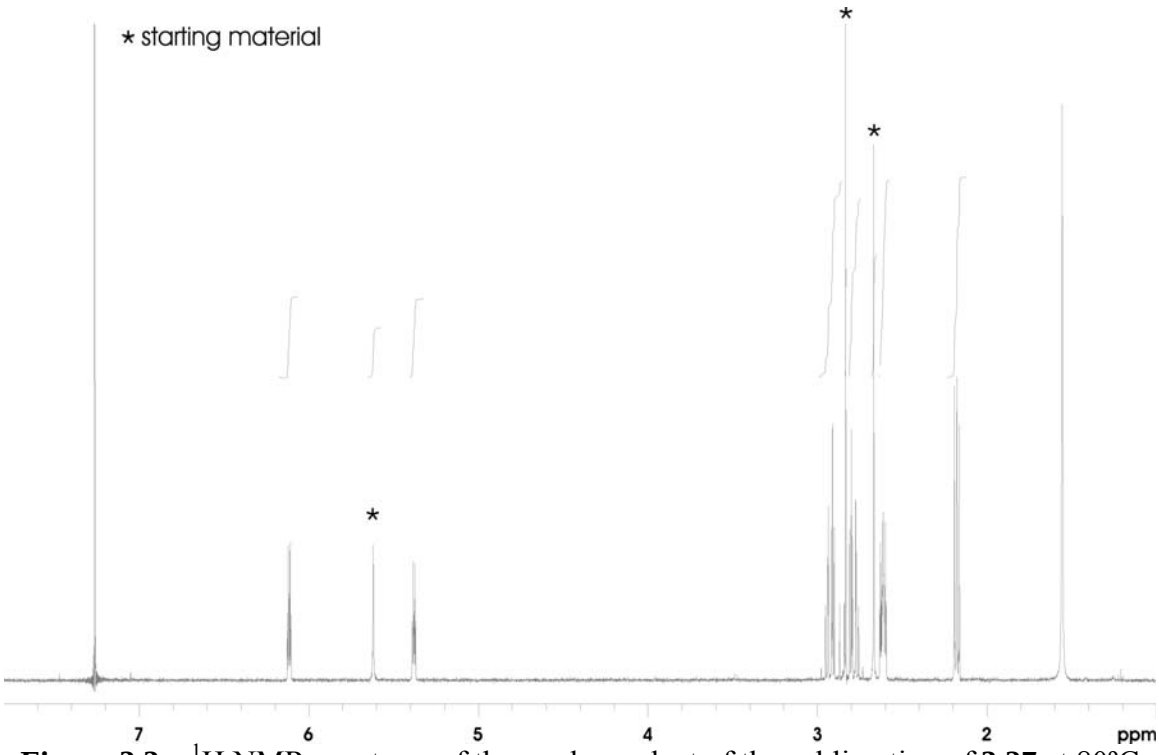
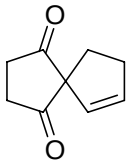


Figure 3.3 – ^{1}H NMR spectrum of the crude product of the sublimation of **3.27** at 80ºC (peaks arising from starting material, **3.27**, are labelled; all others correspond to degradation product **3.36**)

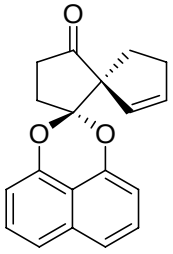
The separation of **3.27** from the degradation product could not be achieved using chromatography on silica. The degradation product is less symmetric than ketone **3.27** as judged by examination of the ^{1}H NMR spectrum and was tentatively assigned as the olefin rearrangement product **3.36** on the basis of 1D and 2D (COSY and HSQC) NMR data. The olefinic protons (δ_H 6.12 and 5.38) display allylic coupling to the allylic methylene protons (δ_H 2.61), which then couple to protons (δ_H 2.18) on a further methylene carbon. The protons (δ_H 2.95-2.76) adjacent to the ketones are also non-symmetric. In the absence of a pure sample of **3.36**, the proposed structure could not be supported by mass spectral analysis, but compounds with similar features were obtained in later work (**3.37**, **3.53** and **3.83**) which support this assignment. The sensitivity of ketone **3.27** to sublimation conditions meant that recrystallisation remained the method of choice in obtaining pure crystalline **3.27**.



3.36

With an appropriate ketone, **3.27**, now in hand, the key spiroketalisation step could be undertaken. Ragot *et al*. discussed, in their synthesis of spirobisnaphthalenes, the surprising difficulty they encountered on forming a spiroketal with DHN.[29] The authors carried out a survey of different acids for the reaction, under classic Dean-Stark refluxing conditions to remove water, and reported best yields when using triflic acid. Dean-Stark conditions with trace quantities of triflic acid were unsuccessfully attempted to form **3.35** from DHN and **3.27**. This reaction was hampered by the formation of large amounts of an intractable black tar and simultaneous consumption of DHN, which is assumed to be due to oxidative polymerisation of DHN to give melanin type polymers. Even under very rigorously de-oxygenated conditions and with exclusion of light, this side-reaction persisted. In these reactions, **3.27** which was pure by ^{1}H NMR spectroscopy but still slightly grey in colour was used. At a later point (*vide infra*) it became evident that a correlation between the "visual purity" of **3.27** and the degree of tar formation existed. Despite the presence of this black tar, **3.27** and some DHN could be recovered after several days of reflux under Dean-Stark conditions, with no desired spiroketal formed. It was found that use of forcing refluxing conditions (125ºC) led to the formation of the correct product, **3.35** (discussed further below), however, it was contaminated with an impurity (**Figure 3.4** - ^{1}H NMR spectrum of the mixture). The ratio of **3.35** to impurity was around 1:1 in a combined yield of around 20% (assuming the impurity is **3.37**, see below). Separation of the two products on silica or diol solid phases was unsuccessful. ^{1}H NMR data showed that the impurity was similar to the olefin rearrangement product, **3.36**, observed earlier during the sublimation of nonadione **3.27**. The olefin protons displayed a similar coupling pattern and COSY and HSQC data were also similar. Comparison of the integrals of the olefinic protons of both products to the naphthalene protons suggested that both products were *spiro*-ketals with DHN. The impurity was therefore tentatively assigned as olefin rearrangement product **3.37**. Since the impurity

was not obtained in pure form, further data to confirm this assignment (eg mass spectral analysis) could not be obtained.



3.37

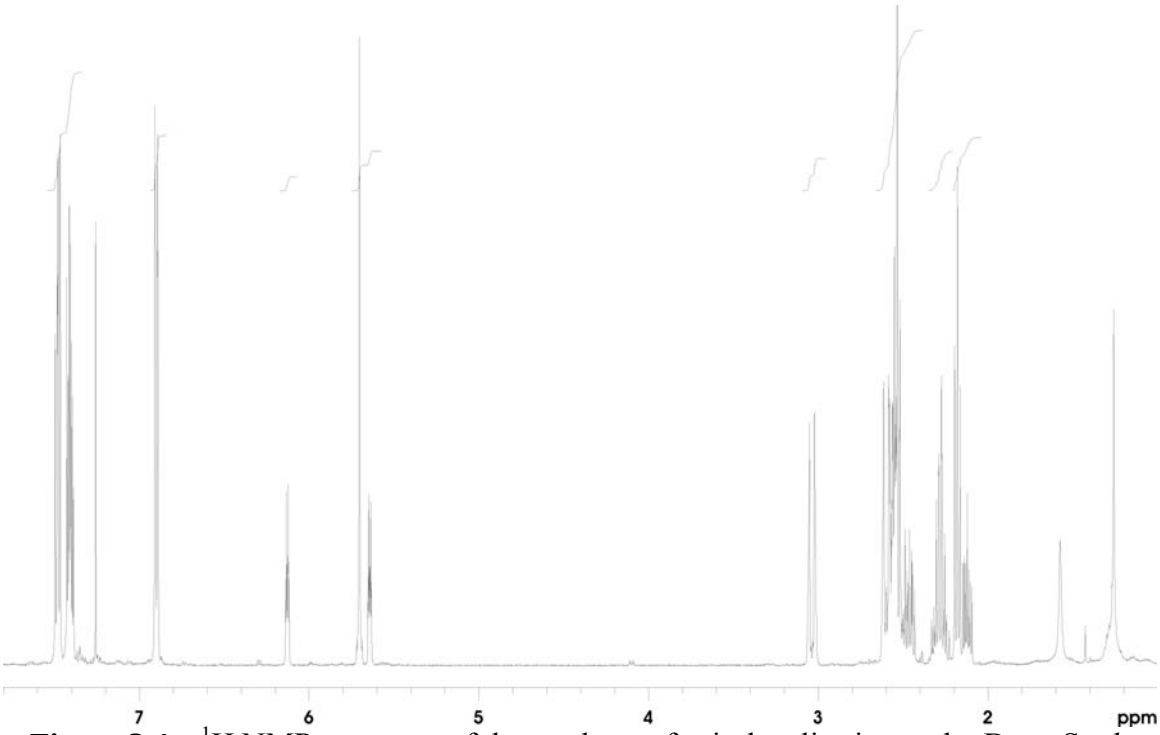
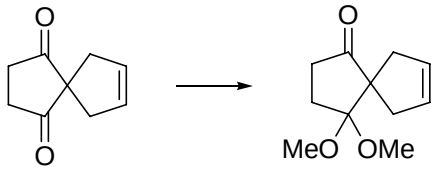


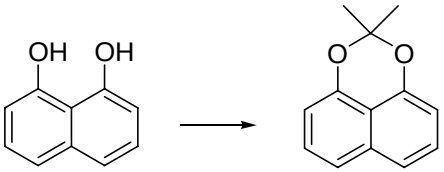
Figure 3.4 – ^{1}H NMR spectrum of the products of spiroketalisation under Dean-Stark conditions (125ºC)

The use of activated molecular sieves as an alternative means of water removal was briefly examined, however no **3.35** was formed and only starting materials were recovered. Alternative, milder methods to form spiroketal **3.35** were found, utilising an acetal exchange method as described below.

It was suspected that the difficulties in forming **3.35** were largely due to inefficient removal of water, which is required to push the reaction equilibrium towards **3.35**. An acetal exchange reaction was therefore considered, allowing the crucial removal of water to be carried out during formation of a ketal from lower-value intermediates. If "simple acetals" of either **3.27** or DHN could be made (**Scheme 3.18**), it might then be possible to carry out an acetal exchange process to generate **3.35** cleanly.



3.27 **3.38**

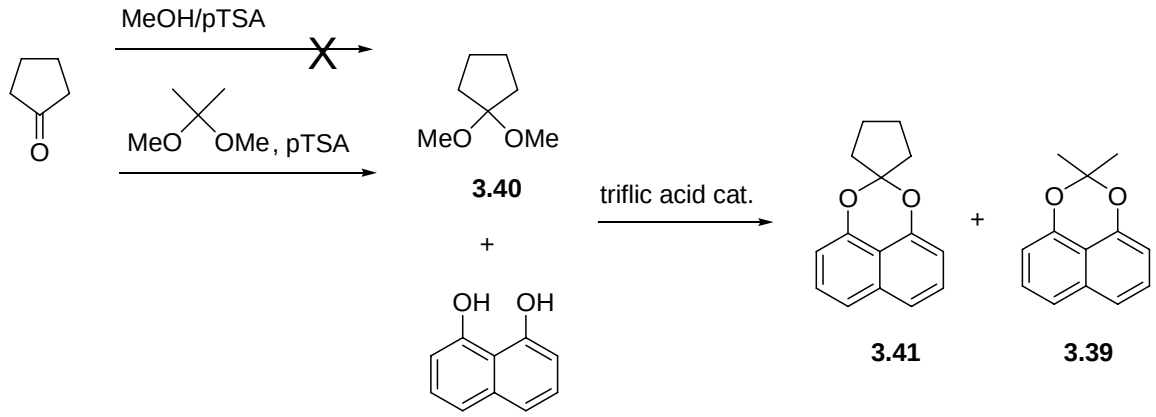


3.39

Scheme 3.18 – Proposed "simple ketals" of **3.27** and DHN, for the purposes of acetal exchange

In order to examine the plausibility of such an approach, a model system was initially examined and cyclopentanone was chosen as a simple model for ketone **3.27**. Formation of **3.40** (**Scheme 3.19**) was attempted using pTSA catalyst in methanol with anhydrous $MgSO_4$ added as dehydrating agent but resulted in no reaction. The use of an acetal exchange to form **3.40** was then examined, using the commercially available, inexpensive dimethoxy-ketal of acetone. By refluxing cyclopentanone in 2,2-dimethoxypropane with pTSA as acid catalyst, the formation of **3.40** proceeded cleanly. Purification of **3.40** was attempted on both deacidified silica and by distillation, however the product proved to be too unstable for purification by these methods. Therefore, after washing to remove pTSA, and removal of most of the 2,2-dimethoxypropane *in vacuo*, the acetal exchange of crude **3.40** was undertaken. Using triflic acid as a catalyst, the acetal exchange of an excess of **3.40** with DHN proceeded smoothly to give **3.41** (~ 28% yield). Variable amounts of

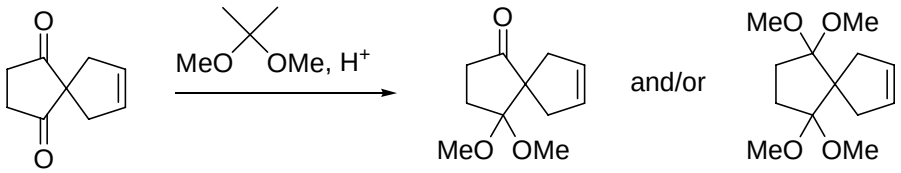
acetonide **3.39** (see below) were also formed, due to the presence of 2,2-dimethoxypropane as an impurity in **3.40**, and the separation of **3.39** from **3.41** was not possible.



Scheme 3.19 – Cyclopentanone model system for acetal exchange

This model system demonstrated both the plausibility of using acetal exchange to generate spiroketals of DHN, and the utility of acetal exchange with 2,2-dimethoxypropane to avoid the need to efficiently remove water.

This model system was extended to form **3.38**, using refluxing 2,2-dimethoxypropane and pTSA as catalyst. In the case of **3.27**, two ketones are present and therefore the possibility of both ketones forming ketals giving **3.42**, could not be ignored (**Scheme 3.20**). This result would be problematic because acetal exchange of **3.42** with DHN could potentially lead to two DHN spiroketals forming. Pleasingly, however, ^{1}H and ^{13}C NMR data suggested that the correct product, **3.38**, had been formed, and no evidence for the formation of **3.42** was observed. As with cyclopentanone, after washing the crude product, **3.38**, and the removal of almost all 2,2-dimethoxypropane *in vacuo*, the acetal exchange reaction was attempted without further purification.



3.27 **3.38** **3.42**

Scheme 3.20 – Possible products of acetal exchange with 2,2-dimethoxypropane

The acetal exchange reaction of **3.38** with DHN successfully gave **3.35** on heating (45ºC) in toluene with triflic acid catalyst (**Scheme 3.21**). The product was fully characterised by ^{1}H (**Figure 3.5**), 2D and ^{13}C NMR spectroscopy and high resolution mass spectrometry.

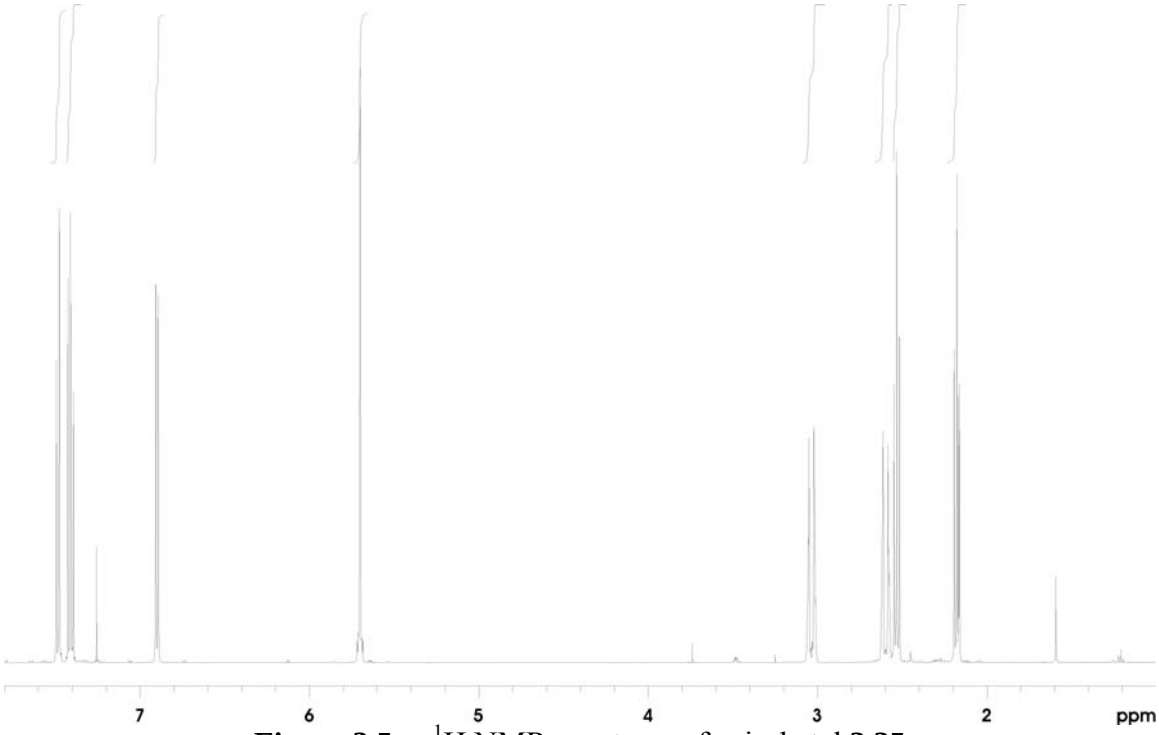
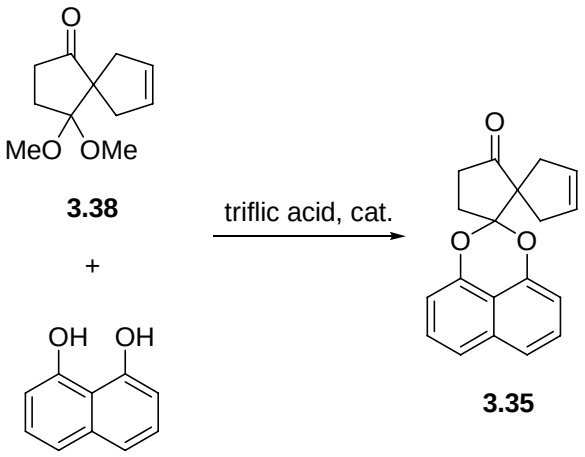


Figure 3.5 – ^{1}H NMR spectrum of spiroketal **3.35**

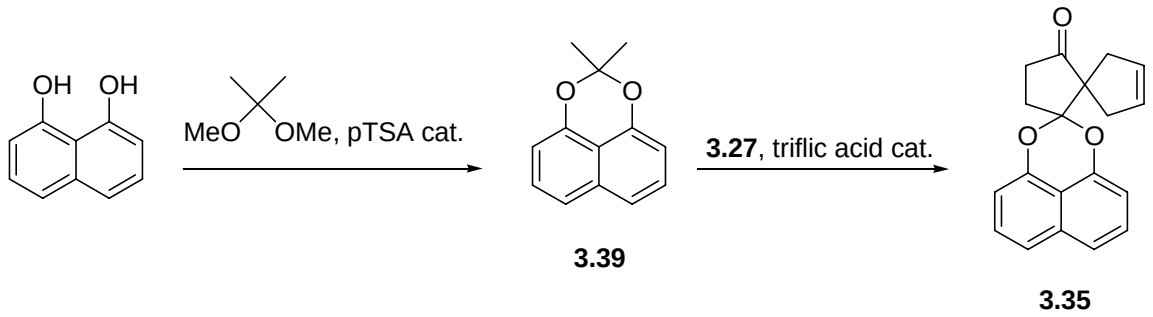
Yields of up to 24% were obtained. This yield is disappointing considering the high yields obtained by Ragot *et al*. (up to 74%). The consumption of DHN was again observed, with black tar formation. The amount of acid catalyst added proved to be important to the reaction outcome, with more than trace quantities required for successful reaction (usually around 0.2 to 0.4 equivalents employed). This result may be due to

proton complexing by DHN reducing the effective quantities of acid catalyst in solution. The sudden darkening of the reaction mixture on addition of acid supports this theory, since the resultant charged DHN delocalised complex would be expected to interact strongly with light.



Scheme 3.21 – Formation of **3.35** by acetal exchange

Whilst this route provided the desired spiroketal **3.35**, it involved using an excess of **3.38** which was not generally recovered in good yield. This is derived from a moderately advanced intermediate, **3.27**, whose two-step synthesis involves use of the expensive Grubbs' II catalyst and is therefore a "high-value" intermediate. An alternative acetal exchange approach, involving formation of a ketal of DHN such as **3.39**, was therefore investigated. DHN can be made in one straightforward step from cheap starting materials making it a lower-value intermediate. The formation of the acetonide of DHN, **3.39**, was found to proceed in good yields of 74% using an acetal exchange reaction with 2,2-dimethoxypropane with pTSA as catalyst. Acetonide **3.39** could be obtained in very pure form by chromatographic purification, first using deacidified silica to remove polar impurities, and then elution through a short reverse phase (C_{18}) pad to remove non-polar impurities. The white crystalline solid produced is stable for several months at room temperature. Increased stability, ease of purification and cheap starting materials make acetonide **3.39** more attractive as an acetal exchange partner than dimethoxy-ketal **3.38**.



Scheme 3.22 – Alternative acetal exchange approach to **3.35**

Acetal exchange of acetonide **3.39** with **3.27** was successfully achieved on heating (45ºC) in chloroform using triflic acid as catalyst to give **3.35** (**Scheme 3.22**). Disappointing yields of around 15% were obtained. Toluene, benzene, DCM and THF were also examined for this reaction. DCM successfully yielded the correct product, although chloroform allowed higher temperatures to be accessed. From all other solvents only starting materials were recovered. The use of pTSA instead of triflic acid as catalyst was also examined, but no reaction was observed and starting materials were recovered.

Despite disappointing yields from this reaction, this route offered access to the crucial intermediate, spiroketal **3.35**. Since most of the precursor ketone **3.27** and variable quantities of the acetonide **3.39** could be recovered, this reaction was used to obtain sufficient **3.35** to continue synthetic efforts towards *spiro*-mamakone A. As this reaction was repeated on a number of occasions, it became clear that the quantities of black tar produced during the reaction varied with the "visual" purity of ketone **3.27**. As mentioned earlier, whilst **3.27** was pure by ^{1}H NMR spectroscopy, different batches ranged in colour from dark to light grey after chromatographic purification. Upon rigorous recrystallisation of **3.27** to a white crystalline solid, acetal exchange reactions were found to occur with almost no black tar formation. This suggests that traces of ruthenium may be responsible for catalysing the oxidative polymerisation of DHN to melanin tar. No further experiments were carried out to confirm this, however. It is interesting to note that no significant improvement of the yield of **3.35** was observed on elimination of melanin production, but acetonide **3.39** was recovered in improved yields.

This suggests that this side-reaction does not compete significantly with the acetal exchange process (an excess of acetonide was employed).

The analytical methods employed during the synthetic efforts towards *spiro*-mamakone A described in this work, deserve brief discussion at this juncture. The *spiro*-mamakone natural products all display a very distinctive UV chromophore, as a result of the dihydroxynaphthalene moiety, as do all of the *spiro*-mamakone synthetic analogues which are described in this thesis. The UV spectra of *spiro*-mamakone A and synthetic analogue **3.35**, shown in **Figure 3.6**, demonstrate the similarity of the UV chromophores of natural and synthetic *spiro*-mamakone analogues.

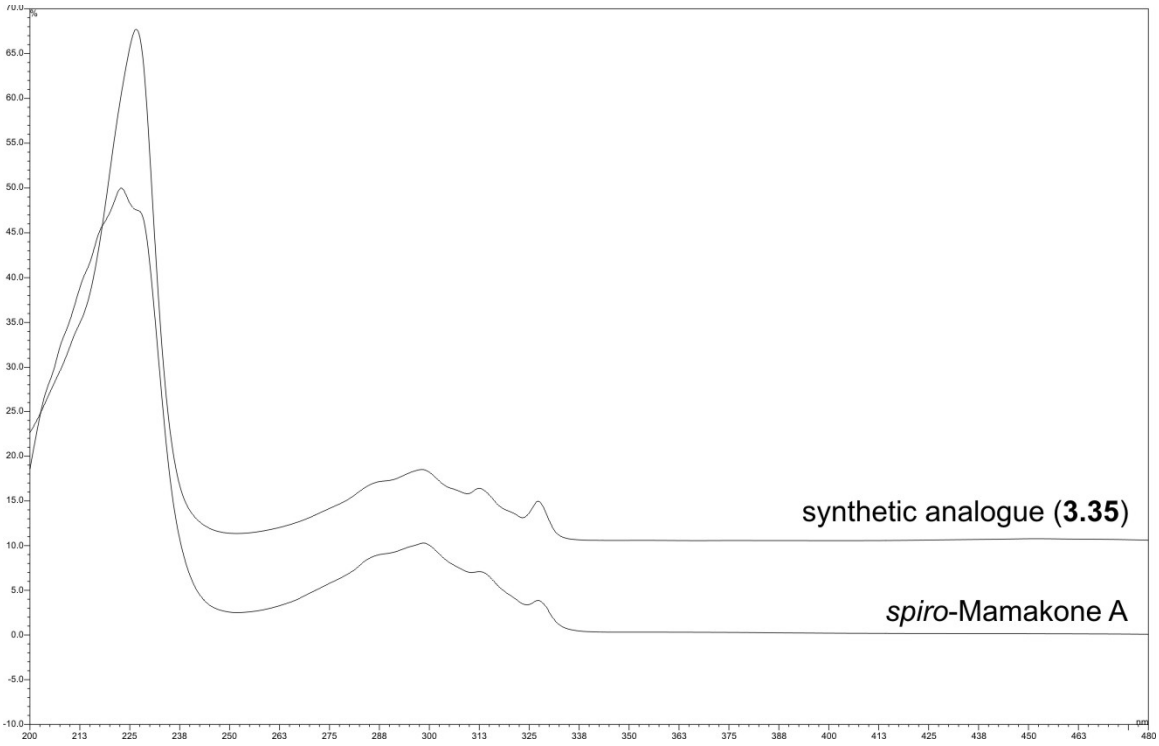
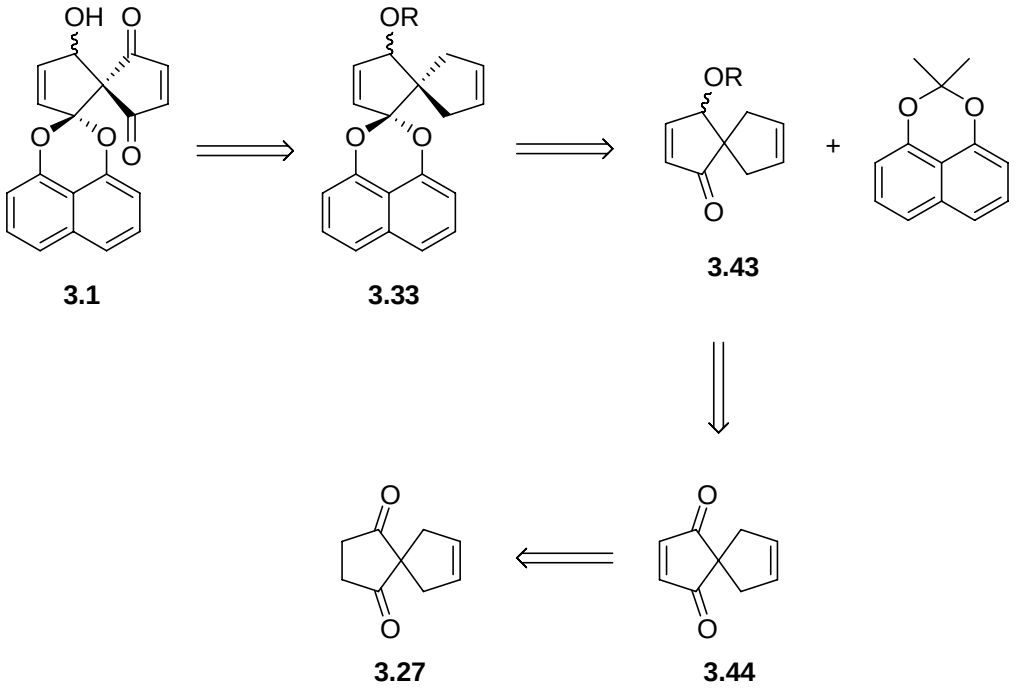


Figure 3.6 – UV spectrum of *spiro*-mamakone A and *spiro*-mamakone synthetic analogue, **3.35**

Monitoring reactions was greatly aided by this distinctive UV chromophore. Reactions could be monitored by classic TLC techniques, using UV (254 nm) and PMA (phosphomolybdic acid) for visualisation. However, HPLC was very useful in monitoring the progress of synthetic reactions, since it allowed ready identification of any new *spiro*-mamakone analogues generated during the course of the reaction.

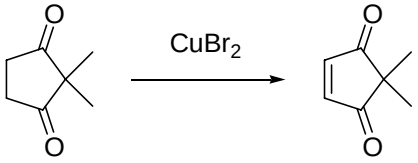
3.2.3 Synthetic investigations of nonadione 3.27

With a method developed to generate a ketal of ketone **3.27** with DHN, two routes towards *spiro*-mamakone A were at this point considered. The first involved taking the intermediate **3.35** through a series of steps to adjust the oxidation pattern of the northern hemisphere. Key steps, as discussed earlier, would include dehydrogenation to give an enone, reduction to an allylic alcohol, and allylic oxidation of the olefin. Investigation of this route is described further below (**Section 3.2.4**). However, an alternative route (**Scheme 3.23**) which was also briefly examined involved synthesis of a more advanced nine-carbon bicyclic system, to which it was hoped the same ketal-formation methodology developed in the synthesis of **3.35** might be applied. This approach has a number of advantages and drawbacks. It allows the crucial spiroketal formation to be carried out later in the synthesis and therefore reduces the number of synthetic manipulations required after the low-yielding spiroketalisation. This minimises the number of small-scale manipulations required. Whilst this ketal has been shown to be remarkably stable under a variety of even acidic conditions in the spirobisnaphthalenes, it is, nonetheless, likely to be the most sensitive functional group in the target molecule, also making late introduction an attractive approach. A possible drawback is the fact that **3.43** is a conjugated ketone, which are known to be more reluctant to form ketals than unconjugated ketones. This may affect the yield or even prevent entirely the spiroketalisation step.



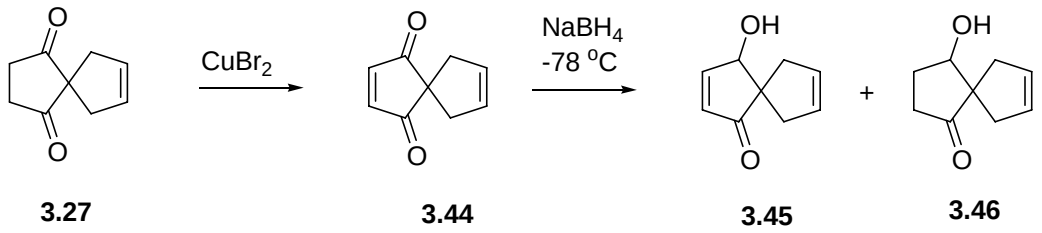
Scheme 3.23 – Alternative retrosynthetic analysis involving dehydrogenation prior to spiroketalisation

The description in the literature of the dehydrogenation of a substrate very similar to **3.27** (**Scheme 3.24**) prompted the investigation of this route. Kreiser *et al.* described the dehydrogenation of 2,2-dimethylcyclopenta-1,3-dione in excellent yields (96%) using copper(II) bromide as oxidant.[46]



Scheme 3.24 – Literature description of dehydrogenation of a cyclopenta-1,3-dione

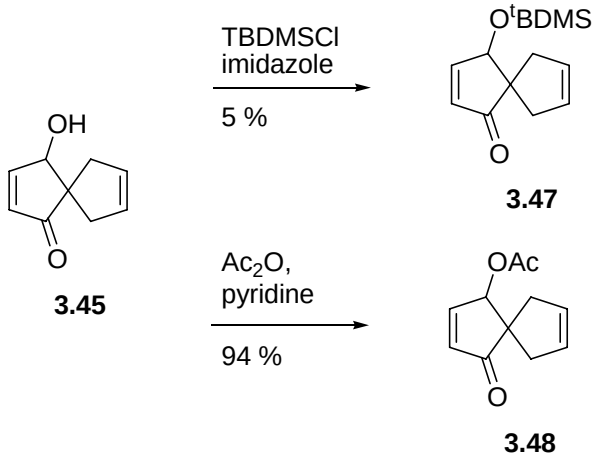
The oxidation of **3.27** was undertaken using identical conditions (**Scheme 3.25**). Pleasingly, the reaction took place in equally good yields (96%) under the same reaction conditions, giving enedione **3.44**. The product was characterised by ^{1}H, 2D and ^{13}C NMR spectroscopy as well as high resolution mass spectrometry.



Scheme 3.25 – Examining the reactivity of **3.27**

Reduction of **3.44** was carried out using sodium borohydride in methanol at -78ºC. Unproductive double-reduction could be avoided by use of an excess of enedione relative to reducing agent, and recovery of starting material. Mixtures of **3.45** (1,2-hydride addition) and **3.46** (1,4-hydride addition followed by 1,2-hydride addition) were obtained in a ratio of around 1.4:1 respectively. Selective reductions to improve this ratio (such as the Luche reduction) were not attempted, however.

Protection of **3.45** (**Scheme 3.26**) was undertaken initially using the ^{t}butyldimethylsilyl protecting group under the classic conditions introduced by Corey and Venkateswarlu.[47] The product was found to be volatile, however, and a yield of only 5% of **3.47** could be achieved. The use of an acetyl protecting group, introduced under standard conditions (acetic anhydride, pyridine) was more successful, yielding **3.48** in 94%. Compounds **3.45**, **3.47** and **3.48** were all fully characterised by NMR spectroscopy and high resolution mass spectrometry.



Scheme 3.26 – Protection of **3.45**

The formation of a spiroketal of **3.48** via trans-ketalisation with acetonide **3.39** was attempted.

The same conditions were employed as those successfully used in the formation of **3.35** (chloroform as solvent and triflic acid catalyst at 45ºC). After one day, ^{1}H NMR spectroscopy of the crude mixture showed that none of the protected allyl alcohol, **3.48**, remained, but significant amounts of the acetonide of DHN were present, suggesting that **3.48** was unstable to the conditions. Trace amounts of a promising compound were present, however, and this product was isolated. The product could only be obtained in good purity after two rounds of chromatography on deacidified silica, which may have been partly responsible for the low overall isolated yield of only around 2%. Characterisation of the product was carried out using ^{1}H (**Figure 3.7**) and 2D NMR spectroscopy (insufficient material obtained for ^{13}C NMR spectroscopy).

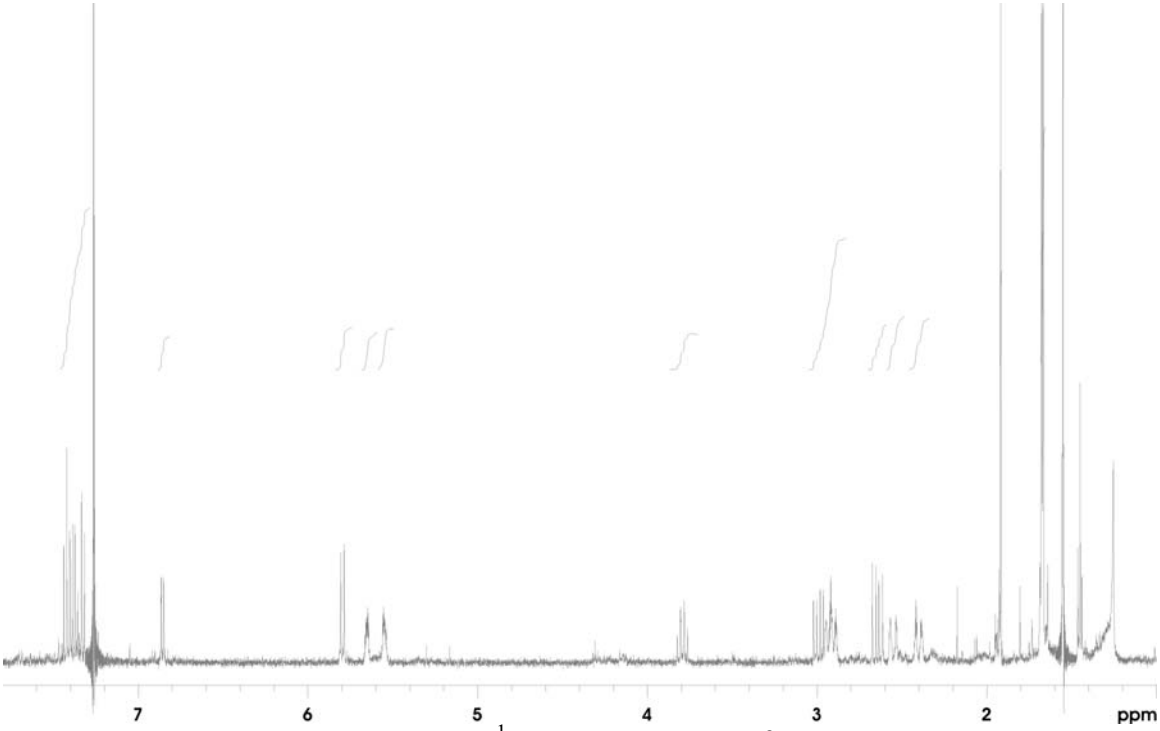
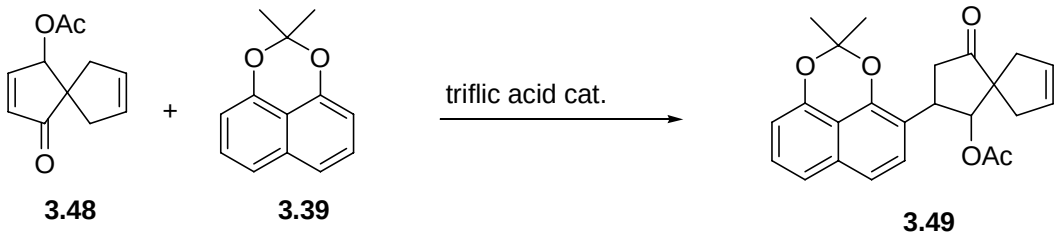


Figure 3.7 – ^{1}H NMR spectrum of **3.49**

Whilst the ^{1}H NMR spectrum showed that aromatic protons as well as protons expected from the nine-carbon bicyclic unit were both present, it was evident that the anticipated spiroketalisation product, acetate protected **3.33** had not been formed. Only five protons

were present in the aromatic region, with the missing naphthalene proton being one of those *ortho*- to a phenol (usually in the region δ_H 6.8 to 7.0). This suggested that substitution at this position had occurred. In addition, only two olefinic protons were present (δ_H 5.65 and 5.55). A COSY NMR experiment showed that the acetate proton (δ_H 5.80) had a correlation to a proton at δ_H 3.79, which itself had correlations to two protons at δ_H 2.99 and 2.64. Three methyl groups were present at δ_H 1.92, 1.68 and 1.67. These data all pointed to the generation product **3.49**, an assignment which HSQC data supported. The molecular formula of **3.49** could not be confirmed by mass spectral analysis, as the anticipated ion was not formed using ESIMS and the sample had decomposed before other ionisation techniques could be attempted. The product is, therefore, only tentatively assigned as **3.49**.



3.48 **3.39** **3.49**

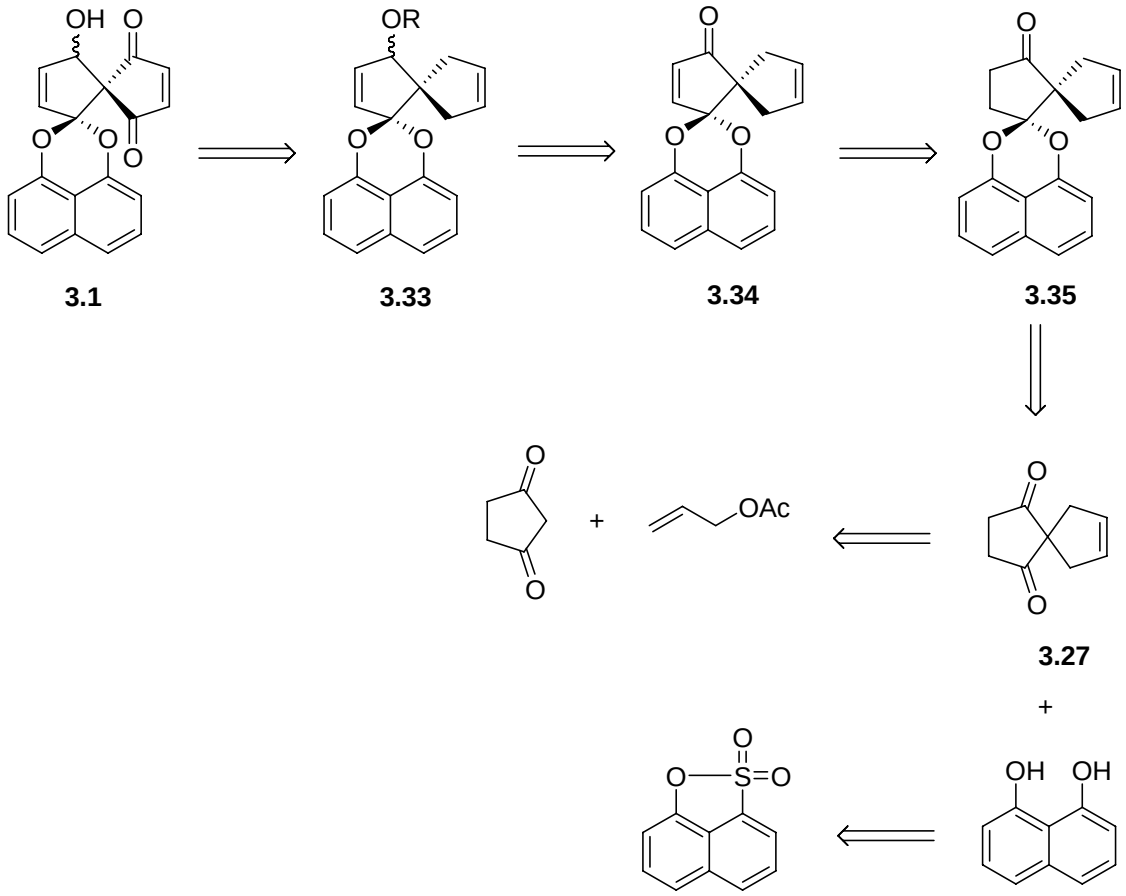
Scheme 3.27 – Unexpected product of spiroketalisation attempt with **3.39** and **3.48**

The formation of **3.49** (**Scheme 3.27**) can be explained by the tendency for enones to undergo 1,4-addition with a suitable nucleophile. In this case, the electron-rich *ortho*-position of the protected naphthalene diol has behaved as the nucleophile. This represents a Friedel-Crafts alkylation reaction, and involves the activation of the enone by protonation by the strong Bronsted acid (triflic acid).

This unanticipated secondary reactivity of enone **3.48** to act as a Michael acceptor in a Friedel-Crafts reaction efficiently competes with the desired trans-ketalisation. Whilst optimisation of this step could have been undertaken, the known reluctance of enones to form ketals led to the abandonment of this alternative strategy to *spiro*-mamakone A.

3.2.4 Synthetic manipulation of spiro-*mamakone intermediate 3.35*

With spiroketal **3.35** in hand, manipulation of the oxidation state of the northern hemisphere could be undertaken. In accordance with the approach shown in **Scheme 3.17** (shown again below), the dehydrogenation of **3.35** was the first synthetic manipulation to be attempted.


OH
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OR
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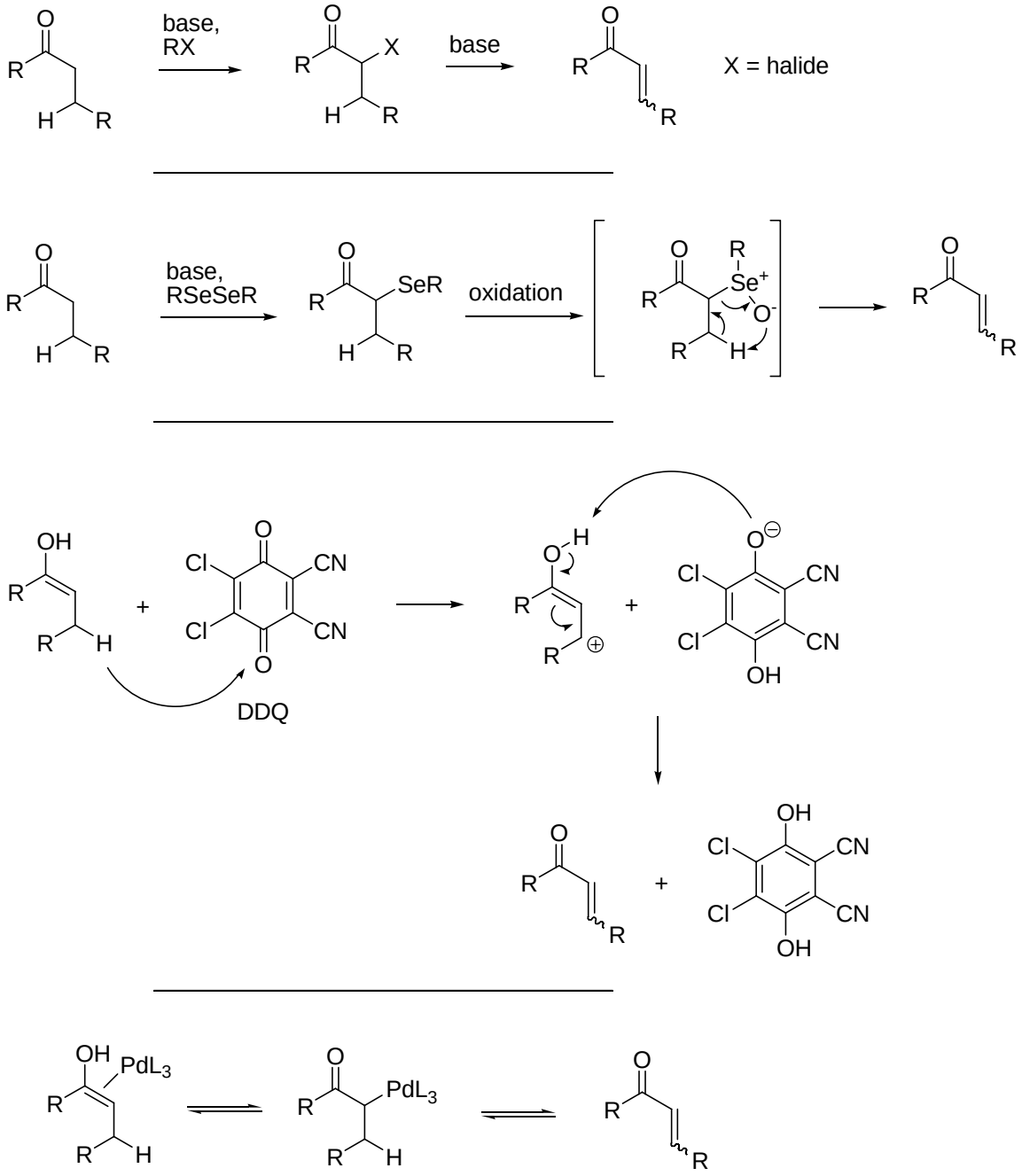
O
O



Scheme 3.28 – General methods for dehydrogenation of ketones

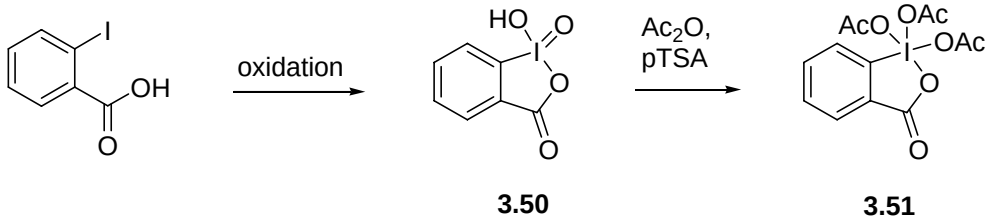
The classical methods involve a two-step halogenation-dehydrohalogenation process, whereby a halide is first introduced α to a carbonyl via its enol or enolate. A base is then employed to promote a dehydrohalogenation reaction to yield an enone. This approach has largely been replaced by an equivalent procedure involving elimination of α-

selenoxides of carbonyls.[49] The use of toxic and expensive selenium reagents detracts from this approach, however. Quinones bearing strong electon-withdrawing substituents such as DDQ can be used to carry out a dehydrogenation directly, in a single step. This is thought to involve an ionic mechanism where hydride abstraction on the substrate enol by the quinone is followed by rapid loss of a proton. The quinone is reduced to a dihydroquinone. Catalytic palladium (II) salts in the presence of cooxidants can also be used to dehydrogenate ketones. This involves the insertion of the Pd into an enol or silyl enol ether, yielding an α-palladium ketone, which eliminates as palladium hydride.

These methods constitute the most common and traditional methods employed for the purpose of dehydrogenating ketones, however other oxidants such as MnO_2 and NiO_2 have been also used successfully. A more recent method devised by Nicolaou *et al*. involves the use of IBX, with or without additives such as *N*-methylmorpholine-*N*-oxide to dehydrogenate ketones or silyl enol ethers and this method will be discussed further below.[50,51,52]

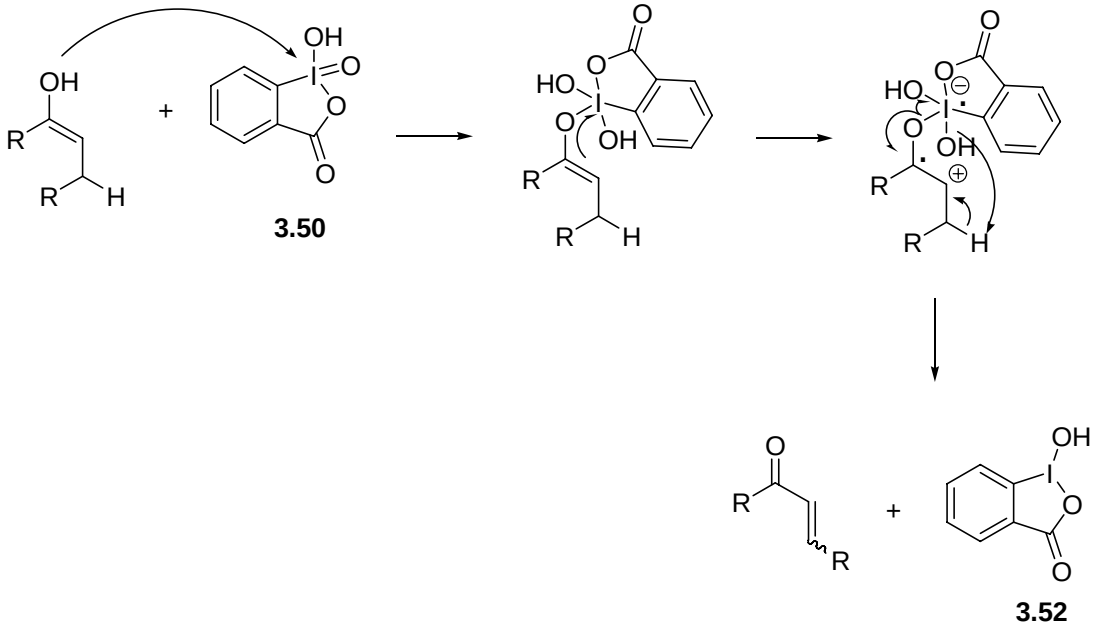
Whilst dehydrogenation via selenium derivative of ketones is a classic method, the use of toxic and unpleasant selenium compounds led to the investigation of some of the other methods mentioned above in the attempt to dehydrogenate **3.35**. DDQ was first examined as oxidant. Ketone **3.35** was mixed with an excess of DDQ (~2 equivalents) under standard refluxing dioxane or benzene conditions.[53] After 24 hours, ^{1}H NMR spectra of the crude reaction mixtures showed that no reaction was taking place.

Next, the very recent dehydrogenation method reported by Nicolaou and co-workers was examined, as this involves readily available IBX, which demonstrates low-toxicity.[50] IBX (*o*-iodoxybenzoic acid, **3.50**) is a hypervalent iodine compound which, despite being known for over a century,[54] has experienced a resurgence of interest very recently. This is attributable to its complete lack of solubility in almost all solvents. A more soluble derivative of IBX, the Dess-Martin periodinane (DMP), **3.51**, became a hugely popular oxidant in natural product synthesis due to its ability to efficiently oxidise alcohols under mild conditions.[55,56]



Scheme 3.29 – Preparation of hypervalent iodine oxidants

The discovery that IBX is soluble in DMSO by Frigerio and Santagostino allowed them to study its chemical reactivity.[57] They found that, like DMP, IBX could act as a mild, efficient and selective oxidant of alcohols to generate aldehydes or ketones and it has since become a popular tool in total synthesis.[56] The reactivity of IBX was extended by Nicolaou *et al*. when they discovered its ability to further oxidise the ketone generated from an alcohol to an enone or even a dienone at elevated temperatures.[50] The proposed mechanism for this process is shown in **Scheme 3.30**. After nucleophilic attack of the enol on the electrophilic hypervalent iodine, a single electron transfer occurs and rearrangement of the resultant radical cation generates IBA, **3.52**, and the enone.

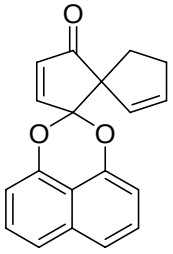


Scheme 3.30 – Proposed mechanism for IBX dehydrogenation of ketones

Further studies on this reaction demonstrated that the active oxidant was a complex of IBX with DMSO which formed at the higher temperatures (> 50ºC) employed during the reaction.[51] A survey of other heteroatom oxides revealed other useful IBX.oxide complexes, in particular IBX.*N*-methylmorpholine-*N*-oxide, which were capable of carrying out the dehydrogenation at room temperature. This methodology has since been applied to the synthesis of natural products, including spirobisnaphthalenes.[58,33]

IBX was generated by oxidation of *o*-iodobenzoic acid using oxone,[59] a method developed by Frigerio *et al.* which involves less toxic and more environmentally benign conditions than earlier methods.[55] The dehydrogenation of **3.35** was attempted using IBX and *N*-methylmorpholine-*N*-oxide (NMO), initially at room temperature. IBX and NMO were first dissolved in DMSO, then **3.35** was added. Ketone **3.35** was not soluble at room temperature in DMSO, so it was added in minimal amounts of DCM (as recommended by Nicolaou *et al.*). After 24 hours, ^{1}H NMR spectroscopy of the crude reaction showed that no reaction was occurring. If the reaction was heated to around 50ºC the desired enone **3.34** (see below) was formed in low yields. The product was contaminated with an impurity which could only be partially separated using chromatography on reverse phase on HPLC. The impurity was assigned as **3.53** by examination of ^{1}H (**Figure 3.8**) and 2D NMR data, high resolution mass spectrometry and by comparison with earlier thermal degradation products, **3.36** and **3.37** (*vide supra*). ^{1}H NMR data showed that the product was related to the product **3.34**, however desymmetrisation of the northern right-hand ring was evident, suggesting that olefin migration had occurred, as seen before. The coupling pattern of the olefinic protons (δ_H 6.01 and 5.63) was very similar to that observed in **3.36** and **3.37**, with correlations to the allylic protons at δ_H 2.58-2.48 and 2.24-2.13. Overlapping with the allylic methylene protons were the non-allylic methylene protons, as evidenced by two distinct methylene carbon signals arising at these ^{1}H chemical shifts in an HSQC NMR experiment. The naphthalene moiety also showed a subtle lack of symmetry with the protons from each aromatic ring appearing at distinct chemical shifts. Mass spectrometry (HRESIMS) indicated that the pseudomolecular ion $[M+H]^+$ had a mass of 291.1022 Dalton, corresponding to a molecular formula of $C_{19}H_{14}O_3$, however trace quantities of **3.34**

present in the sample of **3.53** mean that this result is not completely reliable. The impurity, **3.53**, was present in an approximately 1:1.4 ratio with the product, **3.34** in the crude material.



3.53

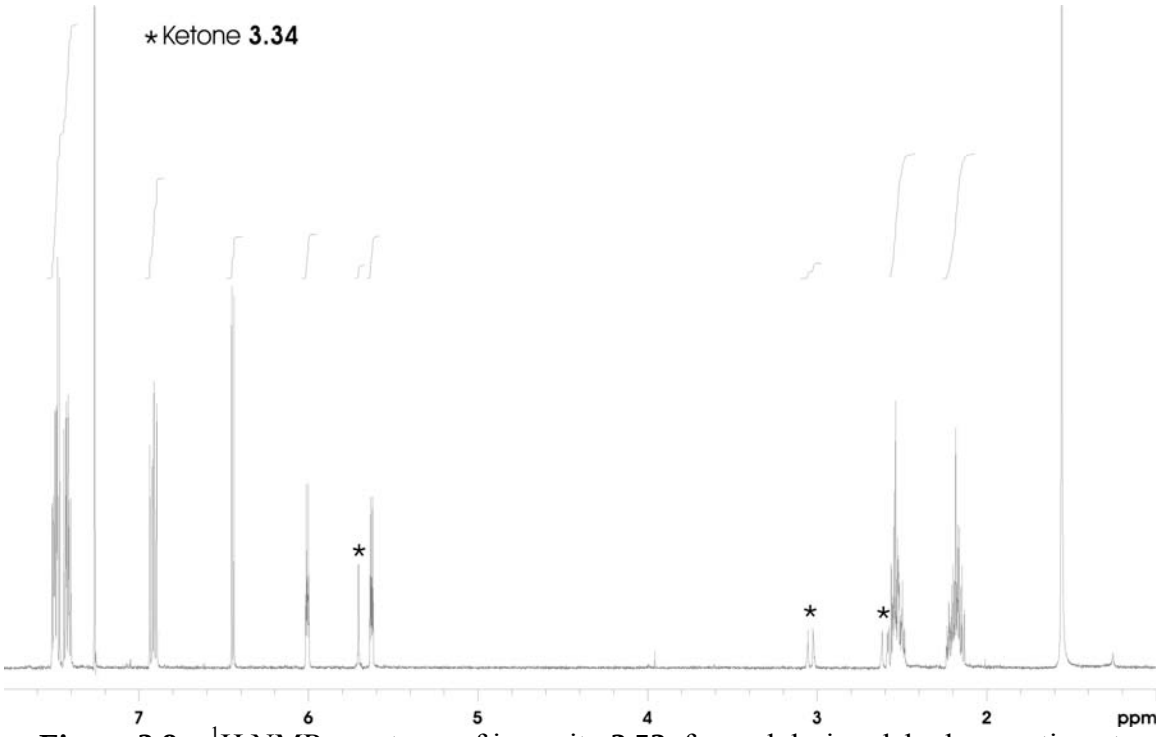


Figure 3.8 – ^{1}H NMR spectrum of impurity **3.53**, formed during dehydrogenation at 50ºC using IBX

If the reaction was carried out at higher temperature (70ºC), ketone **3.35** was found to be soluble in DMSO and was therefore added in the solid state. The reaction was monitored by ^{1}H NMR spectroscopy, and after around 24 hours, a white precipitate was seen to form (IBA, **3.52**). No further reaction was observed with longer reaction time and the reaction was quenched. At this elevated temperature, no impurity was formed and ^{1}H NMR spectroscopy suggested a conversion of around 60 - 70% was achieved. The isolated

yield of **3.34** was usually somewhat lower than this, at around 40%. This may be due to decomposition during chromatographic purification on deacidified silica. The use of diol solid phase did not improve the isolated yield and no further solid phases were investigated. The product **3.34** was fully characterised by ^{1}H (**Figure 3.9**), 2D and ^{13}C NMR spectroscopy and high resolution mass spectrometry.

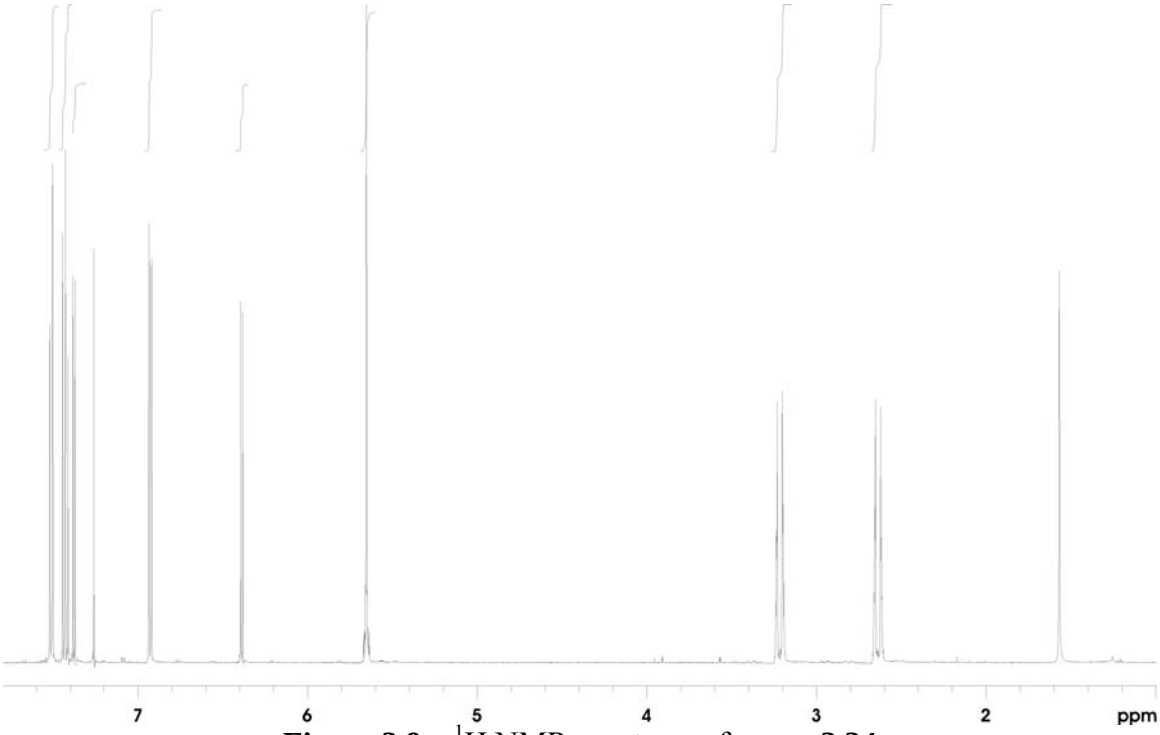
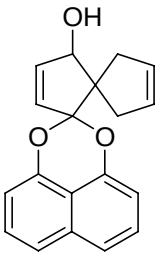
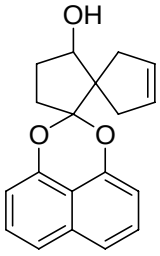


Figure 3.9 – ^{1}H NMR spectrum of enone **3.34**

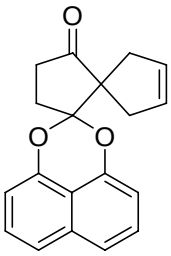
The reduction of the enone to generate allylic alcohol **3.54** was the next step in the synthetic strategy to *spiro*-mamakone A. Initially, simple sodium borohydride and lithium aluminium hydride reductions were attempted, however without success, due to preferential 1,4-addition of hydride to the enone. One equivalent of sodium borohydride gave exclusively **3.55** and **3.35** in a ratio of around 3:2. The alcohol **3.55** is generated by carbonyl reduction of the initially formed **3.35**. Alcohol **3.55** could also be generated by $NaBH_4$ reduction of **3.35**.



3.54



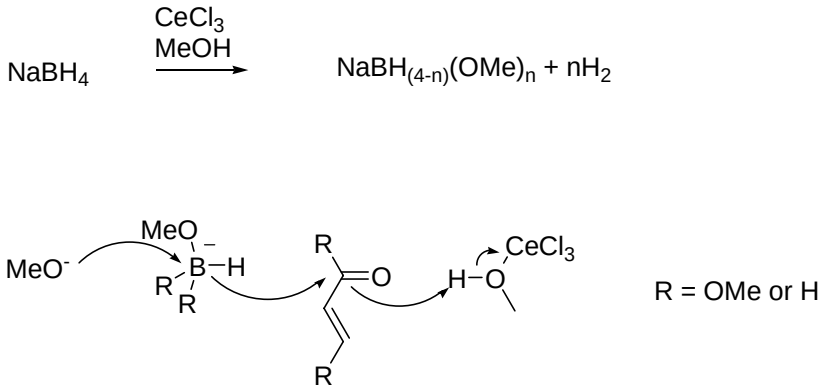
3.55



3.35

The use of 0.3 equivalents of lithium aluminium hydride also resulted in mixtures, but this time, of the desired compound **3.54**, and **3.55**. A ratio of around 1:1 was formed, and these two alcohols proved to be inseparable on deacidified silica.

Examination of the literature revealed that cyclopentenones are known to be particularly prone to undergo 1,4-hydride addition in preference to 1,2-hydride addition.[60] More selective reduction conditions were therefore required to obtain pure **3.54** in good yields. A number of reducing conditions have been reported to be capable of selectively reducing enones to their allylic alcohol,[61] for example 9-BBN, [62] aluminium hydride[63] and DIBAL.[64] However, one of the most popular and commonly used is the Luche reduction.[65] This involves premixing sodium borohydride with non-toxic, lanthanide salt cerium (III) chloride in methanol. The reaction can tolerate significant proportions of water, such that the inexpensive, commercially-available cerium (III) chloride heptahydrate can be employed. This reaction has found extensive utility in natural products synthesis due to its high selectivity and the mild conditions employed.[66,67,68] The mechanism for this reaction is thought to involve the generation of various sodium methoxyborohydrides.[69] These are formed after activation of the solvent methanol by the Lewis acid cerium (III) chloride (**Scheme 3.31**). Since methoxyborohydrides are "harder" reducing agents, they selectively attack the "harder" electrophilic position, which is the carbonyl of the enone.



Scheme 3.31 – Mechanism of the Luche enone reduction

The Luche reduction was therefore applied to the reduction of **3.34** and successfully gave **3.54** in quantitative yield and without contamination with the 1,4-hydride addition products **3.35** or **3.55**. The product was fully characterised by ^{1}H (**Figure 3.10**), 2D and ^{13}C NMR spectroscopy and high resolution mass spectrometry.

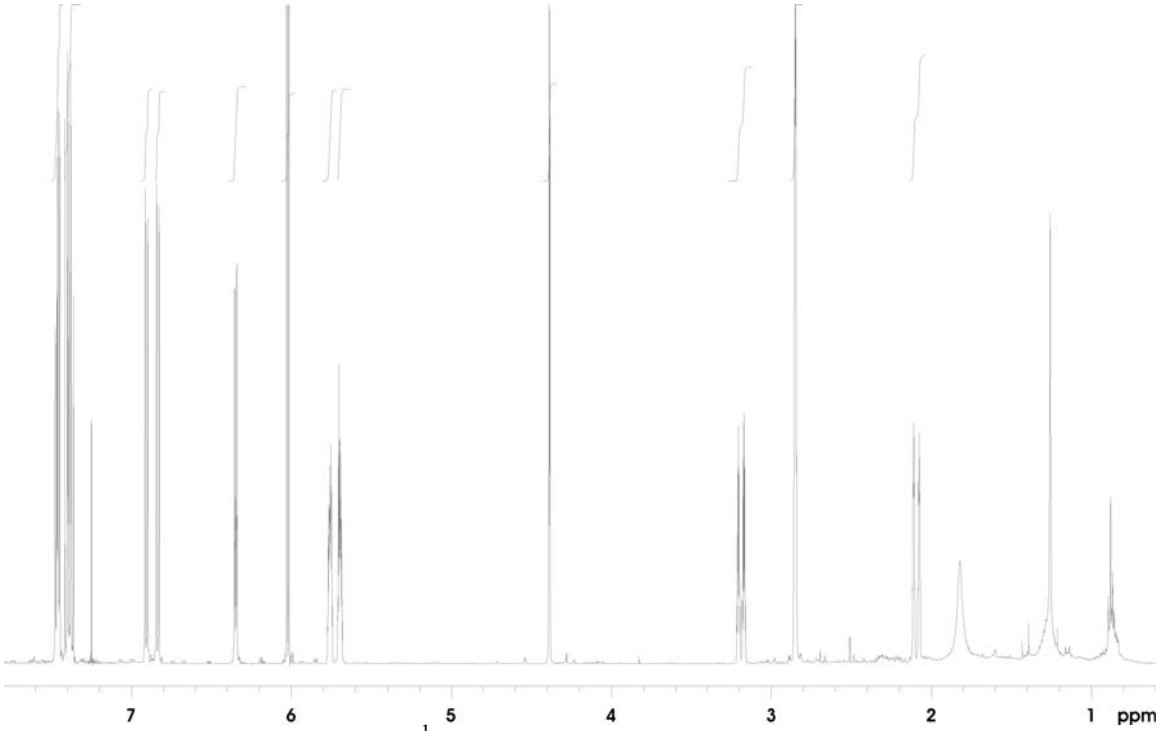
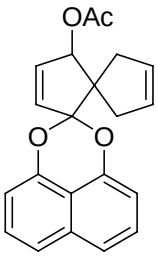


Figure 3.10 – ^{1}H NMR spectrum of allyl alcohol **3.54**

The protection of the allylic alcohol of **3.54** was undertaken before attempting to carry out allylic oxidation to introduce the enedione required by the target *spiro*-mamakone A.

An appropriate protecting group must be removed under conditions to which the final target molecule is expected to be stable. Whilst the spiroketal moiety is known to be relatively stable to acid conditions, the use of a protecting group which could be removed under basic conditions was considered judicious. Both an ester such as the classic acetyl protecting group or a silyl protecting group (which can be cleaved by fluoride sources such as TBAF) would fulfil these criteria. Minimisation of the steric environment around the double bond to be oxidised would maximise the chance of successfully carrying out this crucial, final key step. Therefore the acetyl group was chosen for the protection of the allyl alcohol of **3.54**.

The esterification of **3.54** was carried out under classic conditions, using acetic anhydride in pyridine. This reaction proceeded cleanly to give **3.56** in quantitative yield. The product was fully characterised by ^{1}H (**Figure 3.11**), 2D and ^{13}C NMR spectroscopy and high resolution mass spectrometry.



3.56

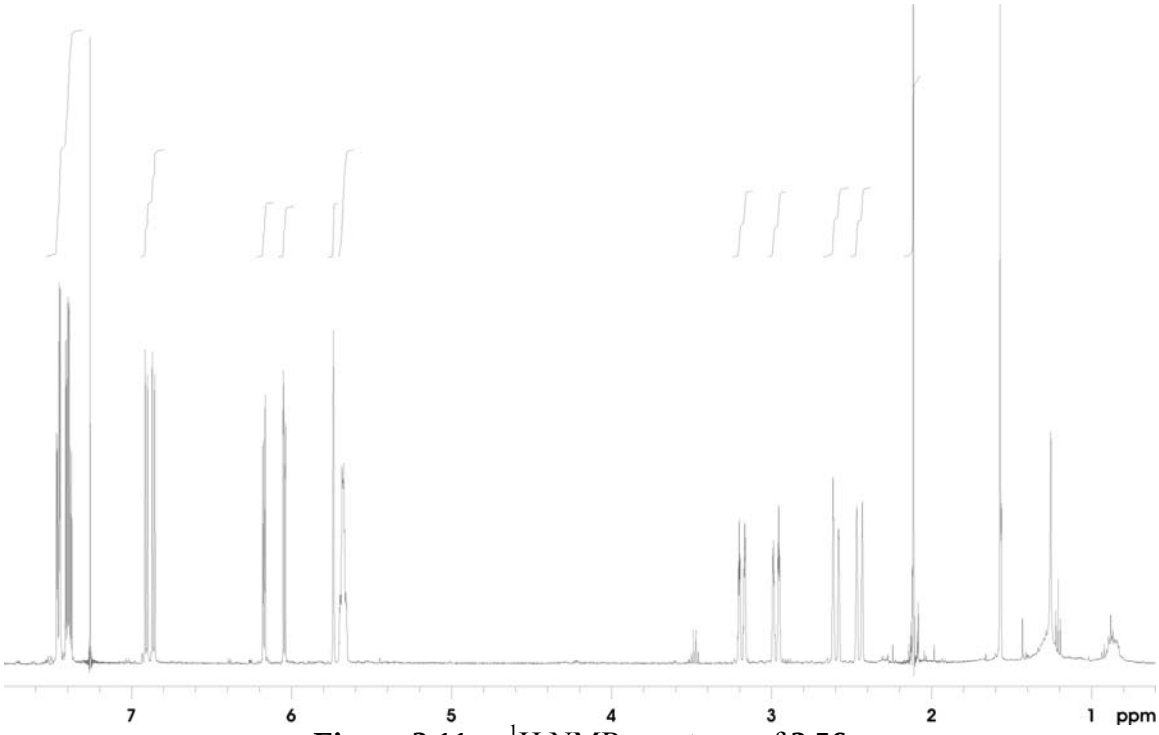


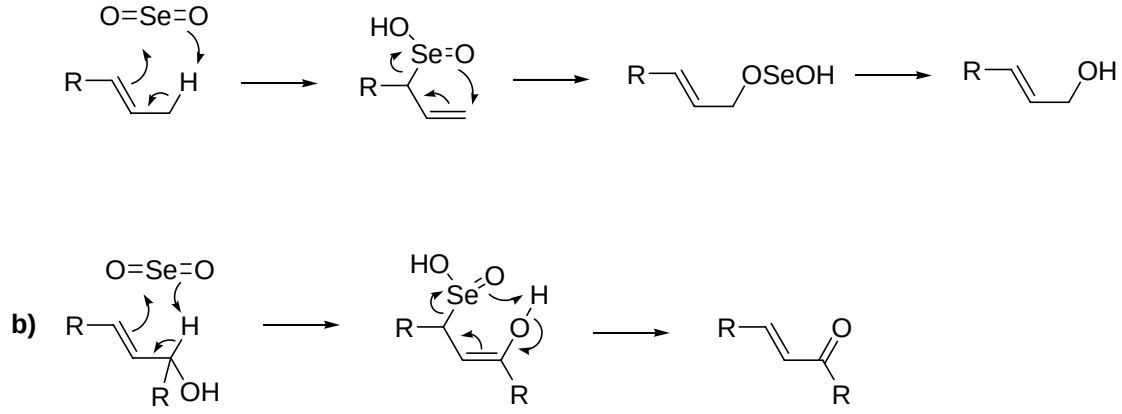
Figure 3.11 – ^{1}H NMR spectrum of **3.56**

With protected **3.56** in hand, the final key functional group conversion required was the double allylic oxidation of the cyclopentene ring to generate the required enedione of *spiro*-mamakone A. There are a large number of different procedures reported for allylic oxidation, and a brief review of some of these is given here.

Allylic oxidations fall into two broad categories; those which generate allylic alcohols and those which give α,β-unsaturated carbonyls directly. In practice, however, many methods straddle both of these categories, giving mixtures of oxidation products or different products under different conditions. Some may also involve double bond migration, or oxidation of the double bond itself. However, whilst a lack of

regiochemical control can plague allylic oxidations, several very reliable procedures are known and the reaction remains of considerable value to organic synthesis.[70]

The classic and still popular reagent for allylic oxidation is selenium dioxide, despite issues of toxicity.[71] It can be used in stoichiometric quantities, or catalytically in the presence of co-oxidants. The most common co-oxidant is ^{t}butyl hydroperoxide, although other oxidants such as sodium periodate and *N*-methylmorpholine-*N*-oxide have been used. This reagent is known to be able to give both allylic alcohols and α,β-unsaturated carbonyls, with a dependence on solvent noted. The generally accepted mechanisms for the reaction are shown in **Scheme 3.32** (allylic oxidation to allylic alcohol is shown in scheme **a**, and further oxidation to α,β-unsaturated carbonyl in scheme **b**).[72,73]

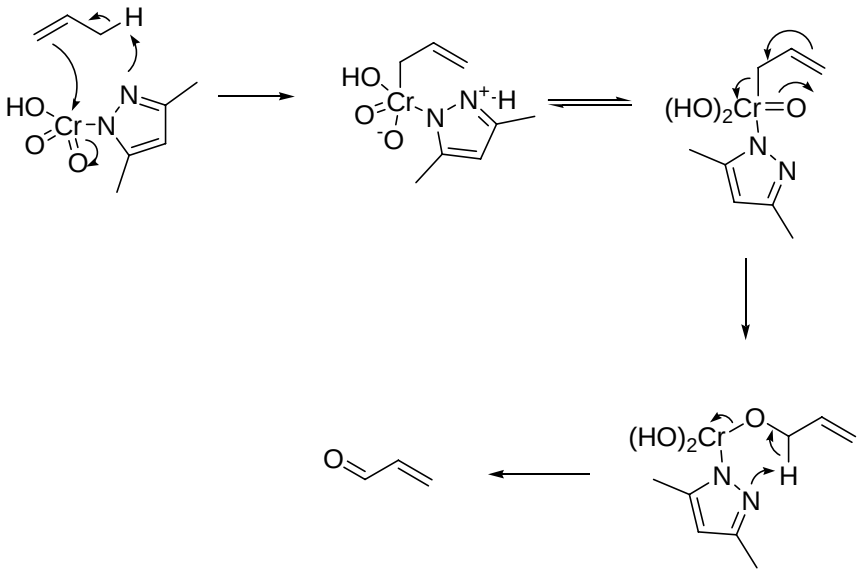


Scheme 3.32 – Mechanisms of SeO_2 allylic oxidation

A once popular allylic oxidation protocol which gives allylic esters is the Kharasch-Sosnovsky reaction.[74] This involves copper-catalysed, radical coupling of an alkene and a carboxyl group, introduced as a peroxyester. Alkene migration can be observed during this reaction. Allylic oxidation using singlet oxygen is also known, and involves migration of the double bond.[75]

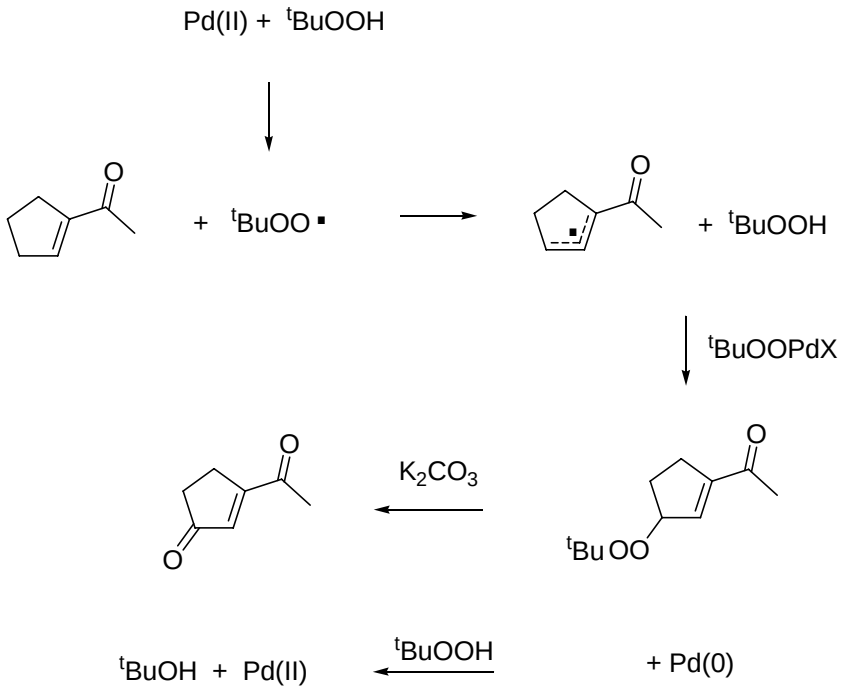
The reaction of certain metal acetates, in particular mercury (II) acetate can generate allylic acetates.[76] Mixtures of products including double bond migration are often observed.[77] The use of manganese (III) acetate has very recently been shown to catalyse allylic oxidation to give enones and even enediones in the presence of ^{t}butyl hydroperoxide.[78] This reaction is believed to proceed by a different mechanism to that of mercury (II) acetate, however.

Chromium based oxidants have been the most popular for allylic oxidation to α,β-unsaturated carbonyls. Early work involved common oxidants such as chromic acid, sodium dichromate and pyridinium dichromate. However, the development by Salmond and co-workers of a chromium reagent specifically designed for allylic oxidations made the reaction far more reliable.[79] The authors found that a complex of chromium trioxide with 3,5-dimethylpyrazole (DMP) offered a massive rate increase for allylic oxidation of steroids in comparison with other chromium based oxidants. They attributed this increase to the ability of the DMP ligand to hasten the removal of the allylic proton and aid attack of the chromium on the alkene (**Scheme 3.33**). This system has found widespread use in the synthetic literature.[80,81]



Scheme 3.33 – Mechanism for allylic oxidation using CrO_3-DMP oxidant system

Palladium-catalysed processes are known to achieve allylic oxidation, however they are often accompanied by varying degrees of isomerisation of the double bond.[82,83] A selective method to oxidise enones to enediones was reported by Yu and Corey, however, using Pearlman's catalyst ($Pd(OH)_2/C$),[84] base and ^{t}butyl hydroperoxide.[85] This is believed to proceed via a palladium-catalysed radical process involving a ^{t}butyl hydroperoxide radical and base-catalysed decomposition of the resulting peroxy ether to generate the carbonyl (**Scheme 3.34**).



Scheme 3.34 – Proposed mechanism for palladium-catalysed enedione synthesis

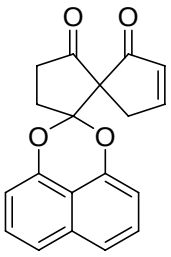
Oxo-vanadium complexes ($VO(OR)Cl_2$, where R is OEt or O^{i}Pr) have been used to carry out allylic oxidations of cyclic enones, to generate enediones.[86] This method has not been adopted by the wider synthetic community, however, possibly due to the requirement of stoichiometric amounts of the vanadium complex.

More recently, Catino *et al.* demonstrated that dirhodium(II) caprolactamate can catalyse allylic oxidation, giving enones and enediones using ^{t}butyl hydroperoxide as stoichiometric oxidant.[87] High catalytic turnover numbers and frequencies, plus mild reaction conditions make this a promising oxidation system.

In the synthesis of *spiro*-mamakone A, the final, key step required is the allylic oxidation of the alkene of **3.33** (or **3.56**) to an enedione moiety (**Scheme 3.17**). Strongly acidic allylic oxidation conditions are precluded by the presence of a spiroketal in **3.33**, although the observed acid stability of the spiroketals of spirobisnaphthalenes suggests that mildly acidic conditions may be tolerated.

Initially, several of the reagent systems described above were screened against the earlier intermediates **3.34** and **3.35**, which were more readily available than the advanced intermediate **3.56**. The oxidants were partially chosen on the basis of ease of availability or preparation (DMP and Pearlman's catalyst were prepared following literature precedures).[88,84] Chromium trioxide and DMP, Pearlman's catalyst and ^{t}butyl hydroperoxide and Catino's dirhodium(II) tetrakis(caprolactam), ^{t}butyl hydroperoxide system were screened against **3.35**. While all three systems gave the same product, they did not all proceed as cleanly. Both the chromium based system and the Pearlman's catalysed oxidation required around 5 days to proceed to a satisfactory conversion (around 60% in both cases, as judged by HPLC analysis). Catino's rhodium based system proved to be the superior method, giving 100% conversion (as judged by HPLC analysis) to the product in around 16 hours. The isolated yields of the product were around 40% using the rhodium system, and around 60% using Pearlman's catalyst. The product from the chromium system was not isolated. The product obtained was fully characterised by ^{1}H (**Figure 3.12**), 2D and ^{13}C NMR spectroscopy and high resolution mass spectrometry. The structure of the product was assigned as the mono-oxidised product **3.57**. It was evident from NMR spectroscopy that an enone had been generated rather than the desired enedione. The position of the enone in the product was assumed to be that shown in **3.57** on the basis that all three oxidation methods are known largely to proceed without migration of the double bond. Whilst no COSY correlation was

observed between the methylene protons at δ_H 2.94 and 2.50 and the down-field enone proton at δ_H 7.55, only very weak correlations were observed between the methylene and the alkene protons in the precursors **3.34** and **3.35**. All other 2D NMR data supported the structure **3.57**.



3.57

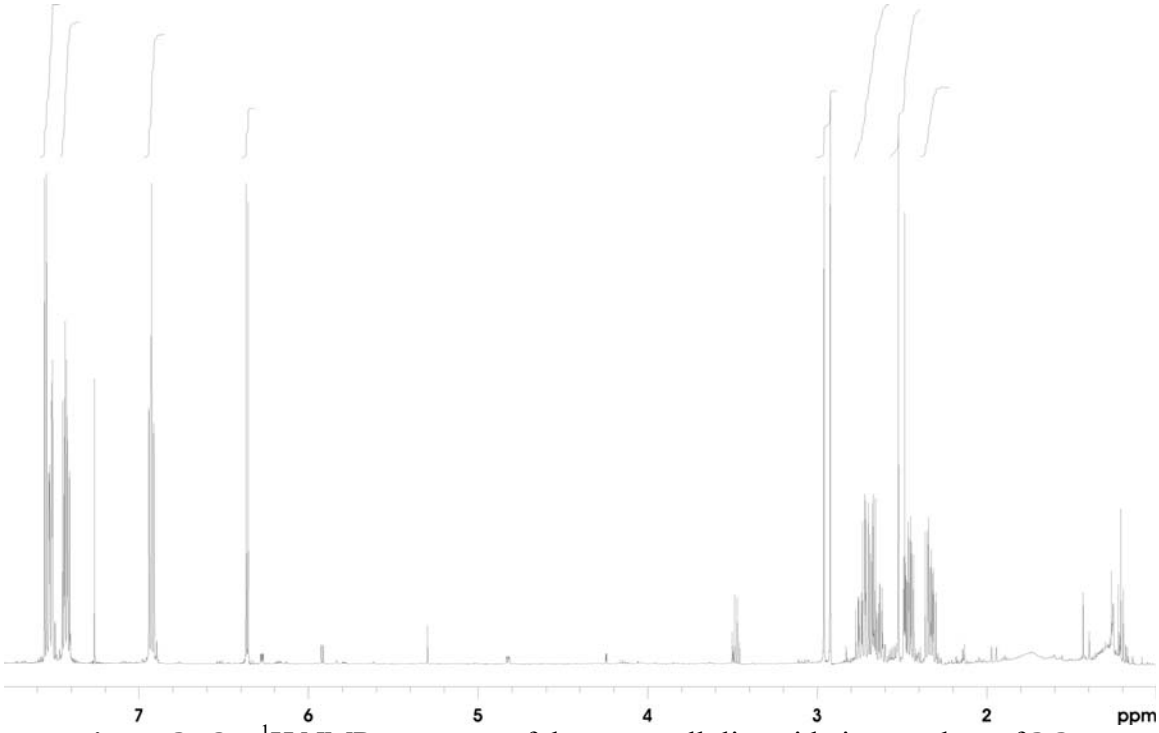
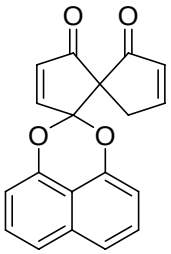


Figure 3.12 – ^{1}H NMR spectrum of the mono-allylic oxidation product of **3.35**

A catalytic chromium system (pyridinium dichromate catalyst and ^{t}butyl hydroperoxide oxidant),[89] and a bismuth catalysed system (bismuth(III) nitrate pentahydrate catalyst and ^{t}butyl hydroperoxide oxidant) were also screened using **3.34** as the precursor.[90] Catino's rhodium catalyst system was also applied to the oxidation of **3.34**. Again, all three systems yielded the same product. The bismuth system proceeded only slowly, reaching around 70% conversion (as judged by HPLC analysis) after 2 days. The chromium

system was more rapid, proceeding to 60% in 1 day and completion after 2 days (as judged by HPLC analysis). The rhodium system again proved to be the best, however, proceeding to around 95% in 1 day. An isolated yield of 32% was obtained from the rhodium system, but no attempts were made to isolate the product from the other two systems (very small scales employed). The product was fully characterised by ^{1}H (**Figure 3.13**), 2D and ^{13}C NMR spectroscopy and high resolution mass spectrometry and the mono-allylic oxidation product **3.58** was assigned. Again, no COSY correlation was observed between the methylene protons at δ_H 3.09 and 2.62 and the down-field enone proton at δ_H 7.56, but all other 2D data indicated structure **3.58** had been formed.



3.58

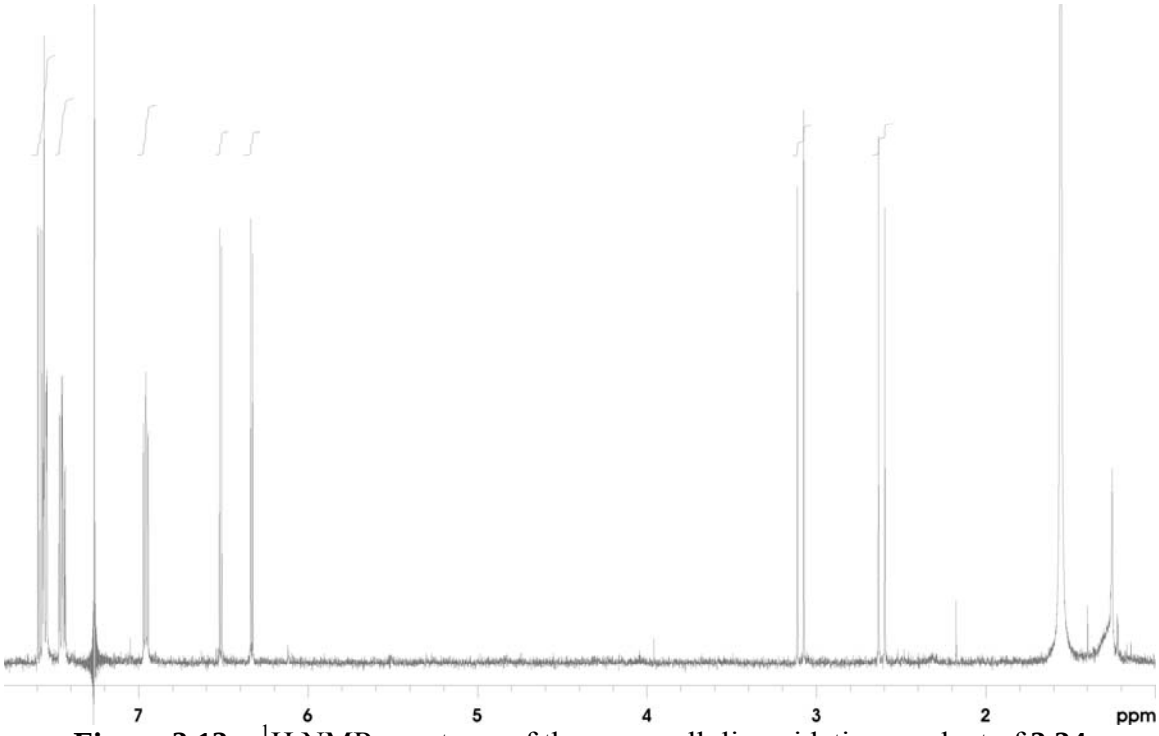


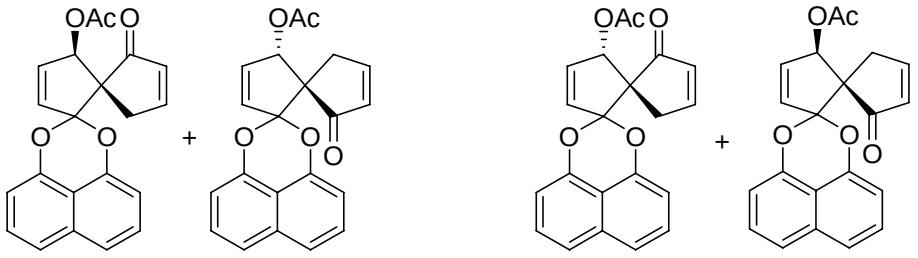
Figure 3.13 – ^{1}H NMR spectrum of the mono-allylic oxidation product of **3.34**

It was evident from these studies that the mono-oxidation of these types of alkenes was relatively facile, with all oxidative systems attempted capable of achieving the oxidation to a greater or lesser degree. The rhodium oxidation system appeared to offer the most complete conversion and shortest reaction times. However, the further oxidation of the enone formed, to generate an enedione required by the final natural product, was unsuccessful. All reactions were monitored for several days after mono-oxidation, with further oxidant (^{t}butyl hydroperoxide, or chromium trioxide/DMP) added but no further oxidation was observed.

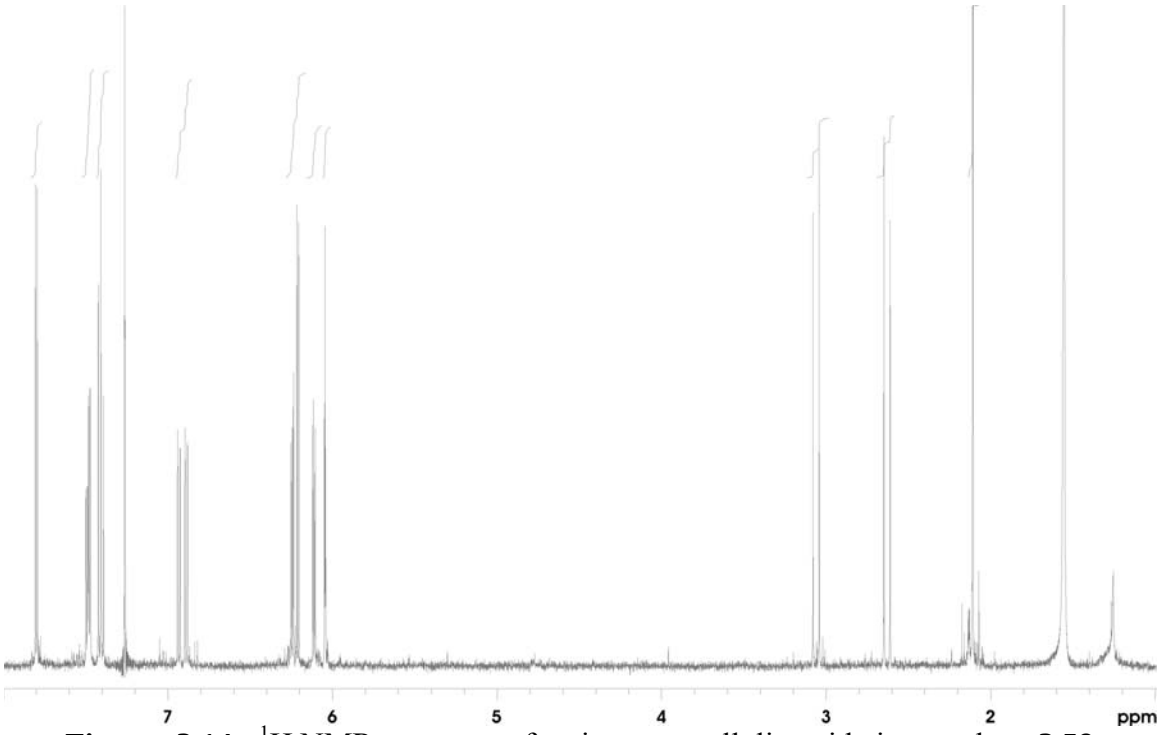
Despite this, the rhodium oxidation system was applied to **3.54** and **3.56** in order to examine the oxidation of these more advanced intermediates. Whilst it may be anticipated that the oxidative conditions employed may be capable of reoxidising the allylic alcohol of **3.54**, its oxidation was still examined. If the allylic alcohol remained unoxidised, the synthetic scheme would be abbreviated by removing the need for protection and deprotection steps, making this route worth examining. However, on oxidation of **3.54** under Catino's conditions complete conversion to the oxidation products **3.34** and **3.58** was obtained (ratio of 2:1, **3.58**: **3.34**) with a 12% isolated yield of **3.58**. This corresponds to complete reoxidation of the allylic alcohol to an enone as well as partial mono-allylic oxidation to generate a second enone. This experiment demonstrates the need for protection of the allylic alcohol before oxidation can be undertaken.

The oxidation of protected allylic alcohol **3.56** was therefore examined next. The rhodium oxidation system was again employed and two products were obtained. These were two mono-allylic oxidation products, with different stereochemistry. This asymmetry arises from oxidation of the alkene on the same side, product assigned as **3.60**, or opposite side, assigned as **3.59**, relative to the protected alcohol. The allylic alcohol, **3.56**, is generated in an asymmetric reduction, such that it is a racemic mixture of two enantiomers. The two diastereomeric enone products generated on mono-allylic oxidation are therefore each a pair of enantiomers. The ratio of the two diastereoisomers was around 4:3 of **3.59** to **3.60**. The oxidation therefore demonstrates a small preference

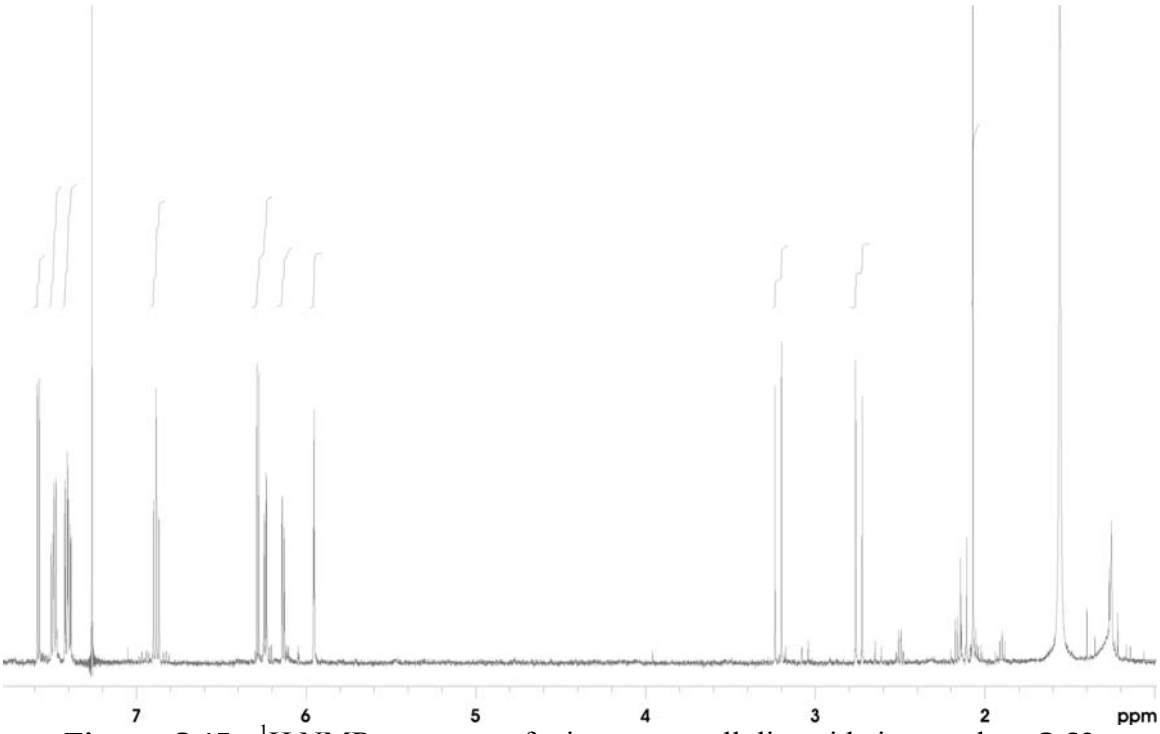
to add oxygen on the opposite side to the protected allylic alcohol; a preference readily explained by long-range steric hindrance. An isolated yield of 25% of **3.59** and 9% of **3.60** was obtained, as well as a further 25% of a mixture of the two products. Both products were fully characterised by ^{1}H (**Figures 3.14** and **3.15**) and 2D NMR spectroscopy and high resolution mass spectrometry. Again, no COSY correlation was observed in either product between the methylene protons and the enone, but all other data were consistent with the structures **3.59** and **3.60**.



3.59 **3.60**



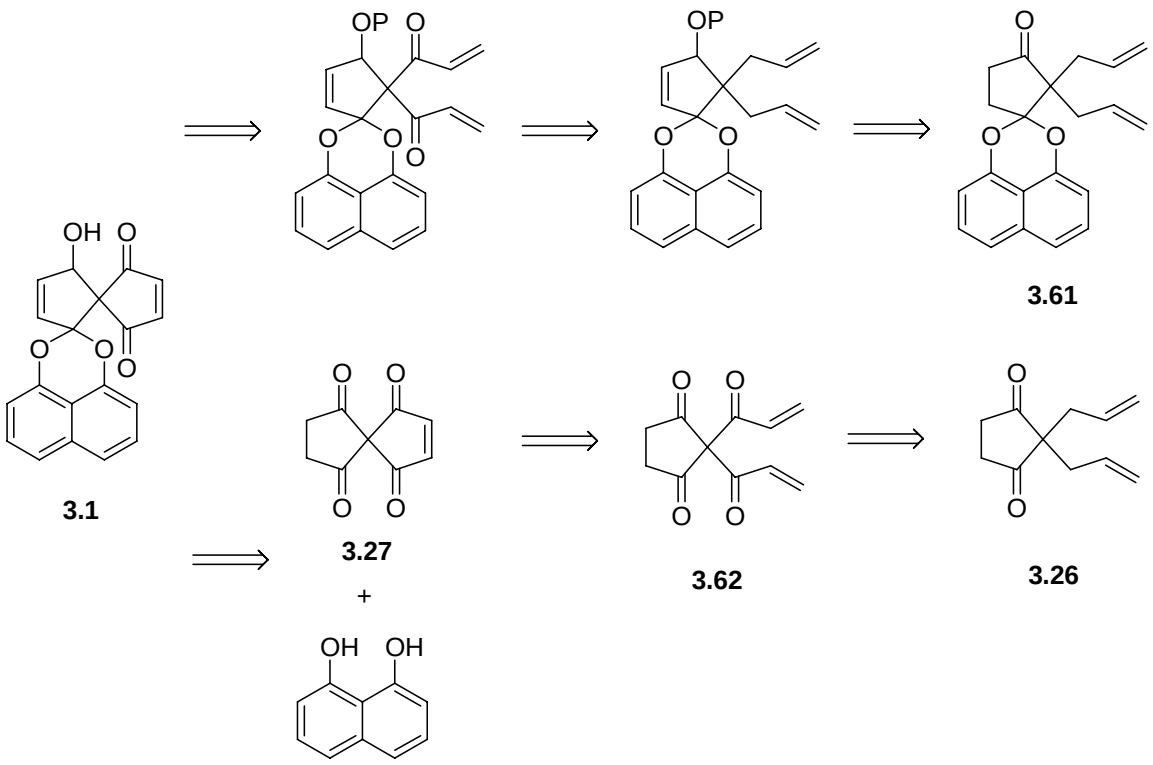
Figures 3.14 - ^{1}H NMR spectrum of major mono-allylic oxidation product, **3.59**



Figures 3.15 - ^{1}H NMR spectrum of minor mono-allylic oxidation product, **3.60**

Products **3.59** and **3.60** are clearly very closely related to the final natural product, *spiro*-mamakone A, and the final key step which remained was the allylic oxidation of an enone to an enedione. Unfortunately, all those reagents which were readily available had been examined and proved incapable of carrying out this conversion. An alternative scheme was examined to try to circumvent this problem (**Scheme 3.35**). As a result of this work, a clue came to light which pointed to an error in the assignment of the structures of all of the products of mono-allylic oxidation, and thereby an explanation for the difficulty encountered when attempting to form an enedione, as discussed below (**Section 3.2.5**).

3.2.5 Investigation of synthetic strategies involving early allylic oxidation



Scheme 3.35 – Alternative route to *spiro*-mamakone A involving late-stage Grubbs' catalysed ring-closing metathesis of two enones.

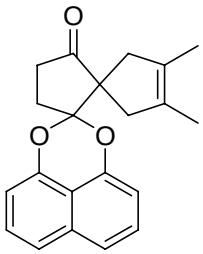
The difficulties encountered in further oxidising the enone generated by mono-allylic oxidation to an enedione was assumed to be due to electronic differences between an alkene and an enone as an allylic oxidation precursor. Re-examination of the literature showed that the oxidative systems employed had varying degrees of precedence in the allylic oxidation of cyclic enones. Catino *et al.* had shown that their rhodium catalysed system could efficiently oxidise cyclic enones to enediones in a number of examples, and Yu and Corey had demonstrated the allylic oxidation of enones exclusively (they did not report oxidation of alkenes). Both the bismuth oxidant system and the pyridinium dichromate system had no precedence for the oxidation of enones, but the chromium trioxide/DMP had been successfully used to oxidise an enone to an enedione.[81] Despite literature precedence for enone to enedione conversions with a number of the oxidant systems employed, an alternative strategy was developed which would allow the

formation of two acyclic enones which could be cyclised to an enedione by late-stage Grubbs' catalysed ring-closing metathesis. This strategy would take advantage of the precursor, **3.26**, of nonadione **3.27**, which contains two allyl groups. The Grubb's catalysed metathesis of enones has been demonstrated by Choi *et al*.[91] The key steps in such an approach to *spiro*-mamakone A would be spiroketal formation, double allylic oxidation and ring-closing metathesis, as well as introduction of the allyl alcohol in the central ring. A number of different routes can be envisioned whereby the order these steps are carried out in differ, with two of the most promising shown in **Scheme 3.35**. In the first route, a ketal, **3.61**, is formed from **3.26**, which is then manipulated to generate a protected allylic alcohol followed by allylic oxidation. Alternatively the allylic oxidation of **3.26** is first undertaken and the enones cyclised to generate the tetraketone **3.62**, which could then form a ketal, followed by manipulation of the central ring to generate the allyl alcohol. Selective reduction of the ketone on the central ring in the presence of the enedione may prove troublesome, making this the less attractive route.

The first of these two routes was therefore examined. The trans-acetalisation of the **3.39** and **3.26** was attempted under the same conditions to those employed for the generation of **3.35** ($CHCl_3$, 40ºC, 2 days). The precursor, **3.26**, appeared to be relatively unstable to the conditions and had degraded (as judged by ^{1}H NMR spectroscopy) after 1 day. As well as starting material DHN acetonide, small amounts of a product clearly related to **3.61** was observed, in the ^{1}H NMR spectrum of the crude sample, but no olefinic proton signals were observed. The product was isolated and fully characterised by ^{1}H (**Figure 3.16**), 2D and ^{13}C NMR spectroscopy, and high resolution mass spectrometry.

High resolution mass spectrometry showed the mass of the pseudomolecular ion ($[M+H]^+$) to be 321.1489 Dalton, indicating a molecular formula of $C_{21}H_{20}O_3$, which corresponds to the molecular weight of the desired compound **3.61**. However, the absence of any olefinic proton signals in the ^{1}H NMR spectrum indicated that **3.61** had not been formed, and therefore that the product was an isomer of **3.61**. The ^{1}H NMR spectrum most closely resembled that of **3.35**, but with an additional singlet corresponding to 6 protons at δ_H 1.66, and the slightly up-field shifted methylene protons (δ_H 2.97 and 2.48) were more broad than in the case of **3.35**. This information allowed

the structure to be tentatively assigned as **3.63**, which was confirmed by examination of 2D and ^{13}C NMR data.



3.63

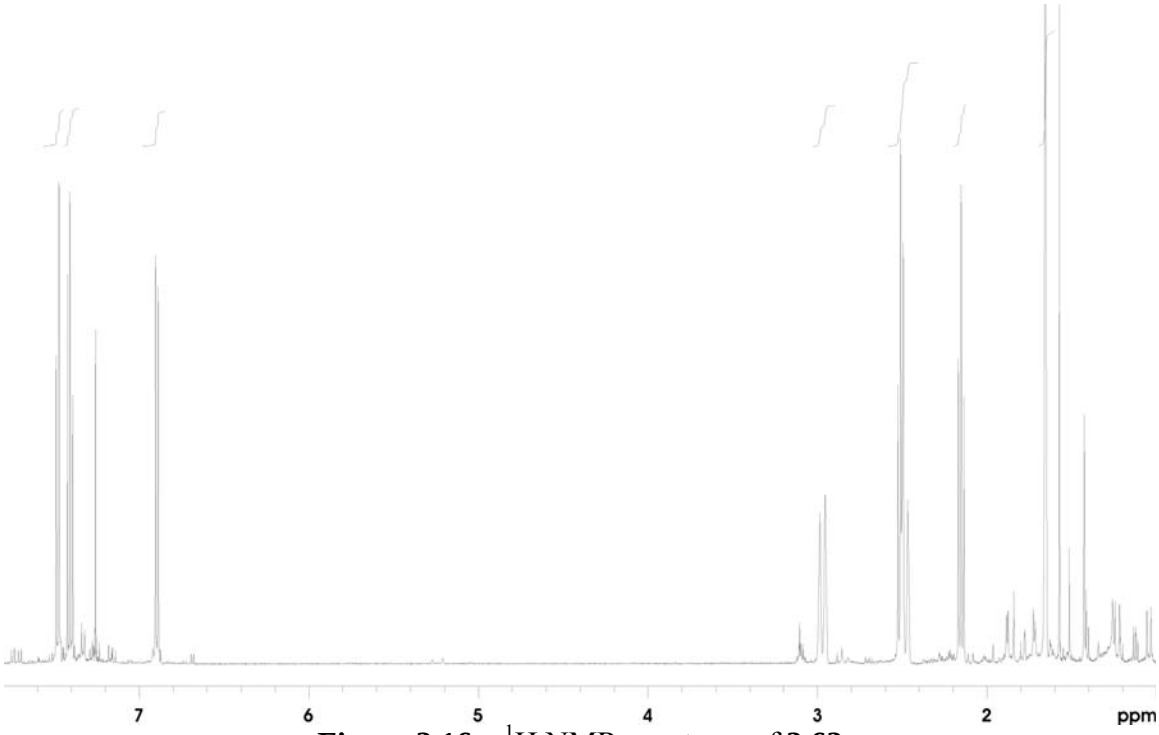
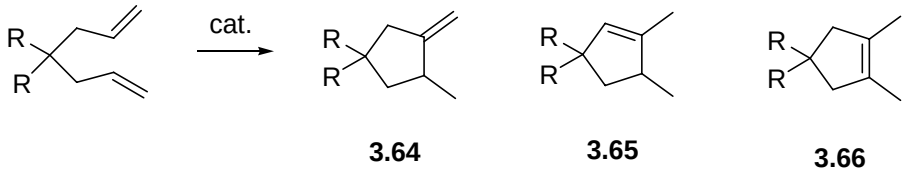


Figure 3.16 – ^{1}H NMR spectrum of **3.63**

This product is clearly derived from the cycloisomerisation of the 1,6-diene, although whether this reaction occurs before or after ketal formation is unknown. Cycloisomerisation of dienes is known to occur under the catalysis of a number of different transition metals.[92] In particular the rearrangement of 1,6-dienes have been shown to undergo cycloisomerisation to give five-membered rings, generally yielding structures of type **3.64** and **3.65**, although occasionally structures of type **3.66** have been encountered (**Scheme 3.36**).[93,94,95] Many transition metals have been shown to promote this type of isomerisation, including palladium.



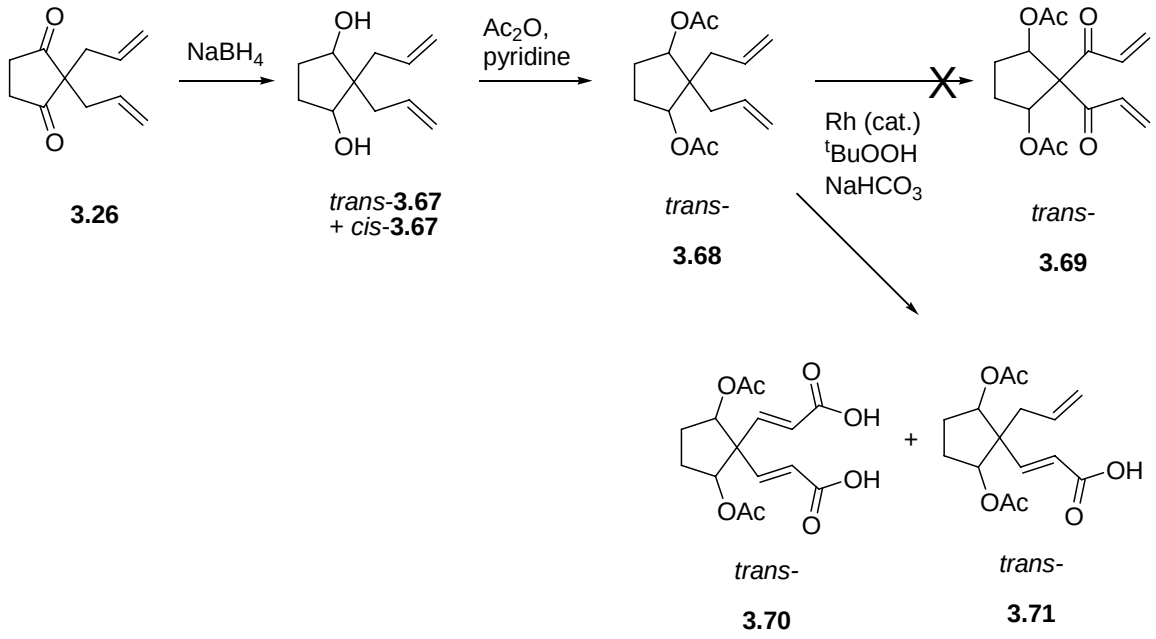
Scheme 3.36 – Cycloisomerisation of 1,6-dienes to yield five-membered rings

This type of reaction has not been observed in the absence of transition metal catalysts. The presence of trace quantities of a transition metal is therefore likely in the trans-acetalisation of **3.26** and acetonide **3.39**. The most likely source is the starting material **3.26**, which is produced via a palladium catalysed dehydration (see **Section 3.2.2**). The diene **3.26** had been purified by chromatography prior to its use in this reaction, however it is possible that trace quantities of palladium impurities remained which were not evident by ^{1}H NMR spectroscopy. As **3.26** is an oil, recrystallisation is not available as an alternative method of purification, and distillation was not considered due to the likely instability of the compound to high temperatures, in particular given it is known to contain traces of palladium metal which may catalyse cycloisomerisation or other degradation pathways. Therefore only repetitive chromatographic methods were an option to further purify **3.26**. Due to the difficulty of monitoring any improvement in purity of **3.26** by ^{1}H NMR spectroscopy, it was decided instead to abandon this route and examine the second route shown in **Scheme 3.35**.

The possibility of carrying out a double allylic oxidation on **3.26** was next examined. The rhodium-based allylic oxidation system was chosen since it had proven the most efficient catalyst in previous studies. After 2 days no reaction was observed and starting material was recovered. This disappointing result led to the examination of the allylic oxidation of a simple analogue of **3.26**, protected diol **3.68**, to see if it could be oxidised.

The reduction of both ketones in **3.26** was readily achieved using sodium borohydride in methanol (**Scheme 3.37**). This gave a mixture of *cis*- and *trans*- diastereoisomers in a ratio of 1.7:1 (isolated yields of 31% *cis*- and 52% *trans*- were obtained). Due to the more simple NMR properties of the *trans*- diastereoisomer, it was decided to proceed

only with this stereoisomer to simplify analysis of later steps. The *trans*-diol **3.67** was next protected with acetyl groups using classic conditions (acetic anhydride in pyridine) to give **3.68**. The allylic oxidation of **3.68** was carried out using the rhodium-based oxidant system. After 3 days, significant quantities of starting material remained (as judged by ^{1}H NMR spectroscopy), however some products had also been formed, so the reaction was stopped and the products isolated.



Scheme 3.37 – Preparation and allylic oxidation of *trans*-**3.68**

Two products were isolated, however both were obtained in very small quantities and only one could be obtained in sufficient quantities (~0.3 mg, ~ 1%) to be fully characterised by ^{1}H (**Figure 3.17**) and 2D NMR spectroscopy. Analysis of ^{1}H NMR data was hampered by residual trace impurities, however a number of key features could be assigned. An "enone" was clearly present as evidenced by two doublet protons at δ_H 7.01 and 5.78. However these integrated in a ratio of 1:1 instead of 2:1 as would be anticipated in **3.69**, suggesting an internal olefinic system. Whilst HMBC correlations observed were minimal due to the small quantity obtained, two carbonyl correlations could be clearly observed. One of these was the anticipated ester correlation of the two acetyl groups at δ_C 171. The other was a correlation from the down-field "enone" proton

to a carbonyl at δ_C 165. This suggested that instead of an enone, an α,β-unsaturated carboxylic acid had been formed. The product was therefore tentatively assigned as **3.70**. Strangely, this product did not ionise readily by electrospray ionisation techniques and the molecular weight of the product could therefore not be confirmed. The second product obtained was not present in sufficient quantity or purity to be characterised by 2D NMR techniques. However, high resolution mass spectrometry showed the mass of the pseudomolecular ion ($[M-H]^-$) to be 295.1178 Dalton, indicating a molecular formula of $C_{15}H_{20}O_6$. This suggests that the product may be tentatively assigned as **3.71**. No attempt was made to repeat this reaction to obtain more material for further characterisation.

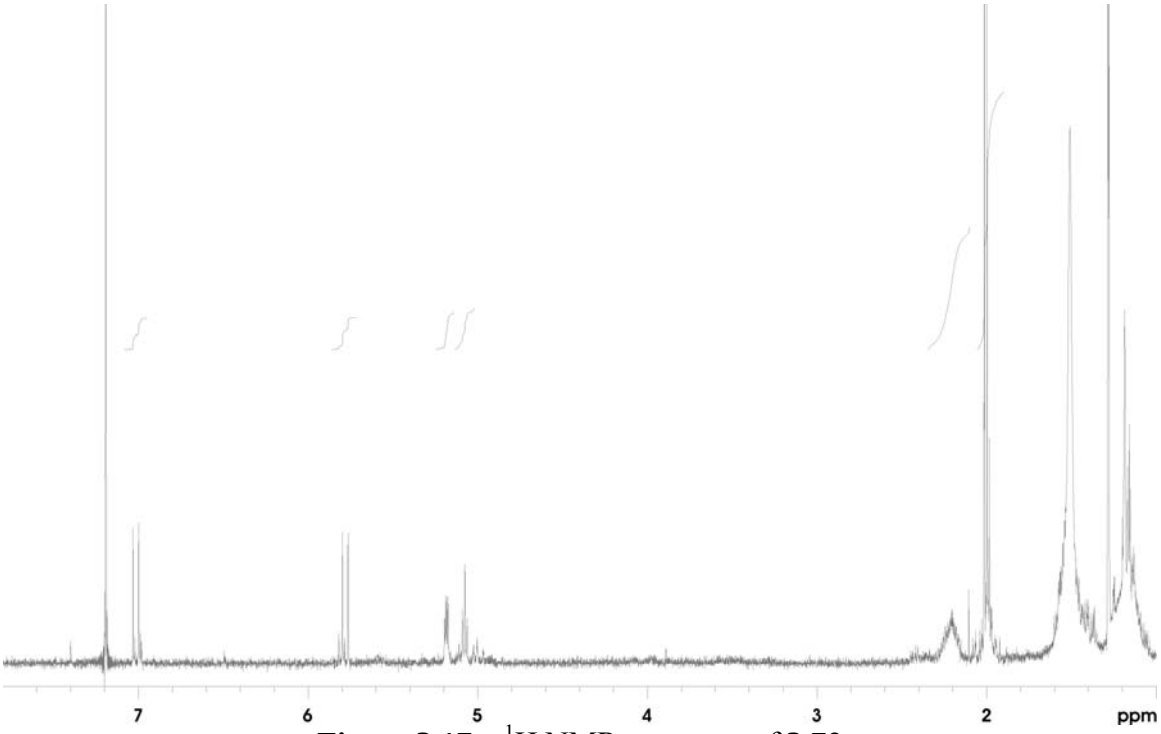
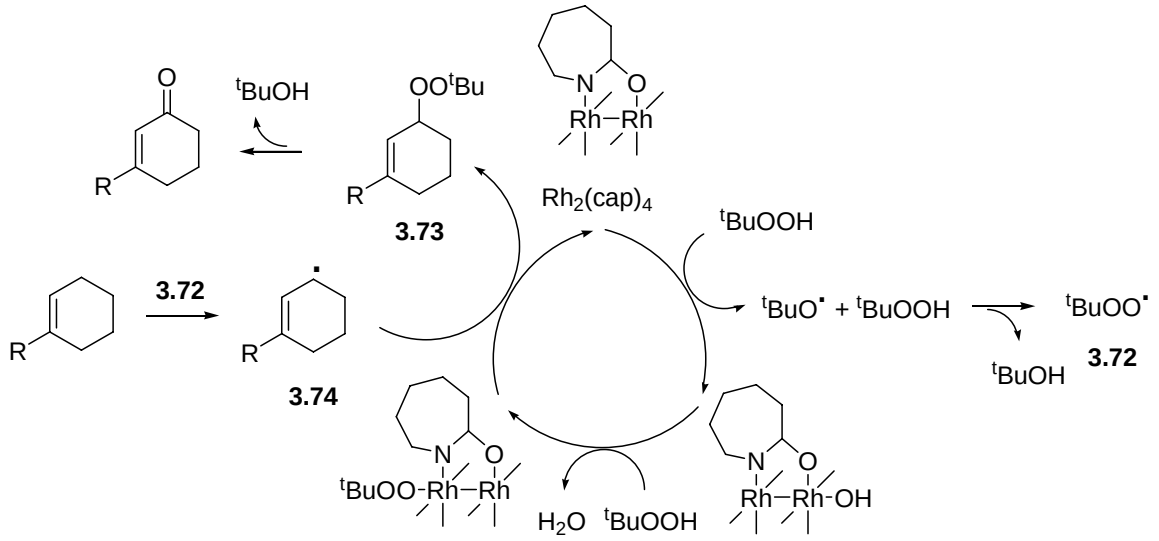


Figure 3.17 – ^{1}H NMR spectrum of **3.70**

The formation of **3.70** (and **3.71**) under the conditions of the allylic oxidation can be explained by examination of the mechanism for this oxidant system, proposed by Catino *et al.* (**Scheme 3.38**).[87] The authors propose that the oxidation occurs via an allylic radical of type **3.74**, generated from a ^{t}butyl hydroperoxide radical; itself generated via a redox process with the rhodium catalyst. The allyl radical then reacts with a dirhodium

^{t}butyl peroxyether complex to generate the mixed peroxide, **3.73**, which decomposes in the presence of base to give the final enone (or enedione). The intermediacy of an allylic radical opens up the possibility of alkene migration in the final product, since the radical is necessarily located over two carbons. Of the eleven examples given by Catino and co-workers, only one was given in which the final product formed via migration of the olefin, however this was probably due to the choice of substrates employed in the published work.



Scheme 3.38 – Proposed mechanism for the allylic oxidation system of Catino *et al.*

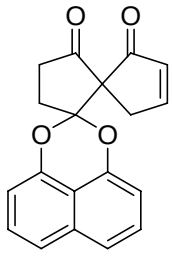
In the oxidation of *trans*-**3.68**, it is probable that once an allyl radical is formed the dirhodium ^{t}butyl peroxyether complex is only able to interact with the carbon furthest away from the *spiro*- centre, due to steric hindrance. This would lead to the generation of a terminal, mixed peroxide which would decompose to an aldehyde. Under the oxidative conditions, the aldehyde is presumably then further oxidised to generate an α,β-unsaturated carboxylic acid (as in **3.71**). If this process were repeated with the second allyl group, a second α,β-unsaturated carboxylic acid would be generated to give the product **3.70**. This is the proposed mechanism for the formation of **3.70** and **3.71** in the allylic oxidation of *trans*-**3.68**.

This experiment showed that this route was not an immediately amenable pathway to *spiro*-mamakone A and was abandoned. The products **3.70** and **3.71**, and the proposed

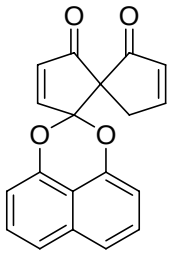
mechanism for their formation showed that the rhodium-based oxidant system was readily capable of causing double-bond migration during oxidation of these *spiro*-compounds. This led to a reassessment of previous oxidations discussed in **Section 3.2.4**.

3.2.6 Reassessment of allylic oxidation reaction

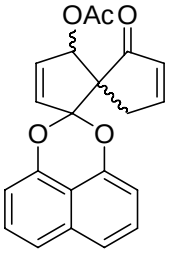
Investigations of the allylic oxidation of acyclic allyl groups drew attention to the possibility of generating oxidised olefins with migration. This led to a reassessment of the structures assigned in **Section 3.2.4** (**3.57** to **3.60**).



3.57

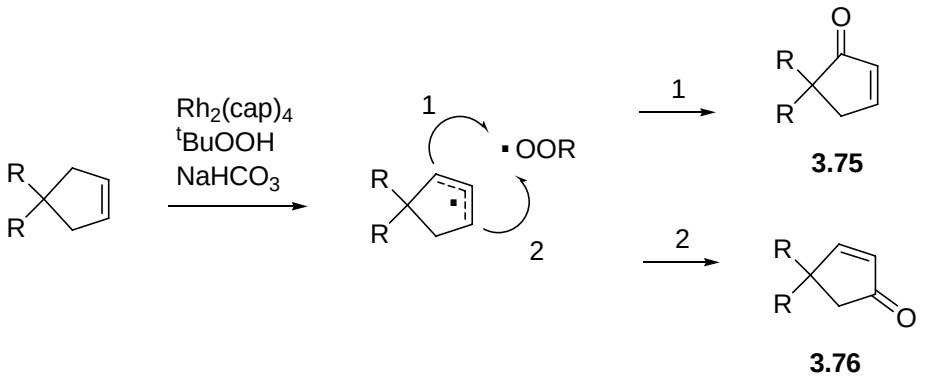


3.58



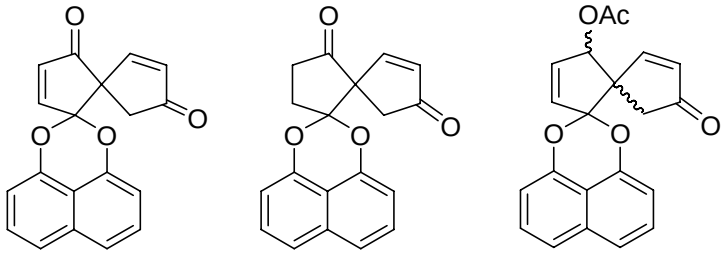
3.59 + **3.60**

In assigning these structures, the possibility of alkene migration was overlooked due to the minimal number of examples in literature studies of the oxidant systems employed. However the allylic oxidation of acyclic olefin **3.68** had shown that in these *spiro*-systems oxygen may be introduced at the less sterically encumbered carbon of the intermediate allyl radical. Two sites, C6 and C8, exist for reaction of this radical with a dirhodium ^{t}butyl peroxyether complex (**Scheme 3.39**), yielding, after base-catalysed decomposition of the mixed peroxides, the enones of type **3.75** and **3.76** respectively. Of these two sites, C6 is much more sterically hindered, due to the *spiro*- centre α to it and therefore C8 is the more likely site of reaction.



Scheme 3.39 – Two possible oxidation pathways

Reaction at C8 of the allyl radicals of **3.34**, **3.35** and **3.56** would lead to the products **3.77**, **3.78**, **3.79** and **3.80**.



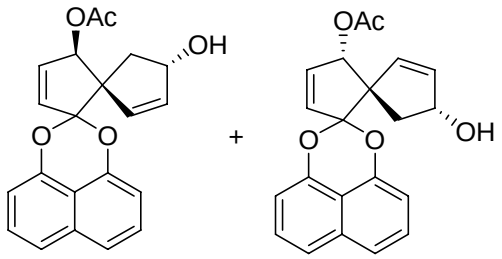
3.77 **3.78** **3.79** + **3.80**

The tentative reassignment of these products allowed a number of both chemical and spectroscopic features to be explained. The difficulty in carrying out further allylic oxidation steps can be readily explained by the lack of an allylic hydrogen in these structures, which makes it impossible to generate an allyl radical for further oxidation of the enone to an enedione. In addition, the lack of even a weak COSY correlation between the down-field enone protons and the methylene groups provided further evidence. However, NMR spectroscopy did not provide proof of these reassignments, since no 2D correlations were observed which could distinguish between the two possible sets of products. Further evidence was therefore sought.

Luche reduction of the enone generated would provide a means to differentiate enones of type **3.75** and type **3.76**. In type **3.75**, the allylic alcohol proton would only couple to an olefinic proton, whereas in type **3.76**, it would couple to both an olefinic proton and two

methylene protons. For this experiment, **3.79** was chosen as the precursor enone. This was as the other enones contained a second ketone, which would be expected to be reduced simultaneously and would lead to mixtures of products. Enone **3.79** contained no other reducible groups and had been obtained in diastereomerically pure form, which would simplify analysis of the reduction product.

The Luche reduction of **3.79** led to one main product, where the reduction of the enone is presumed to have occurred from the face opposite the naphthalene moiety (NOESY correlations to confirm this assumption were not observed). Whilst small amounts of another stereoisomer were observed by ^{1}H NMR spectroscopy of the crude material, this product was not isolated in sufficient purity for full characterisation. The main product was fully characterised by ^{1}H (**Figure 3.18**) and 2D NMR spectroscopy as well as high resolution mass spectrometry. Mass spectrometry showed the mass of the pseudomolecular ion to be 333.1120 Dalton ($[M\text{-}OH]^{+}$), which corresponds to the molecular formula $C_{21}H_{18}O_5$. The observation of the dehydrated ion is explained by the ease of dehydration of allylic alcohols to generate dienes. Examination of COSY 2D NMR data showed that the allylic alcohol (δ_H 4.81) had a correlation to both an olefinic proton (δ_H 6.08) and two methylenic protons (δ_H 2.50 and 2.25). This clearly proved that the enone from which the allylic alcohol was derived was of type **3.76**. All other 2D NMR data supported the structure of the reduction to be **3.81** (mixture of enantiomers).



3.81

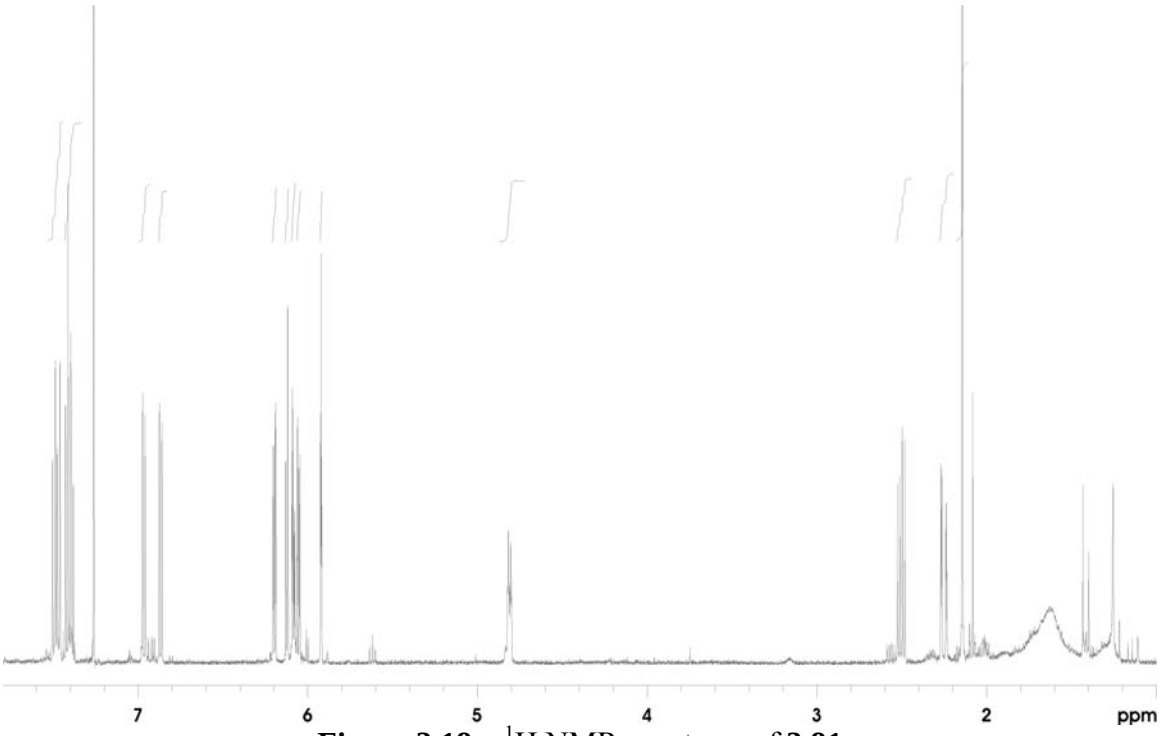


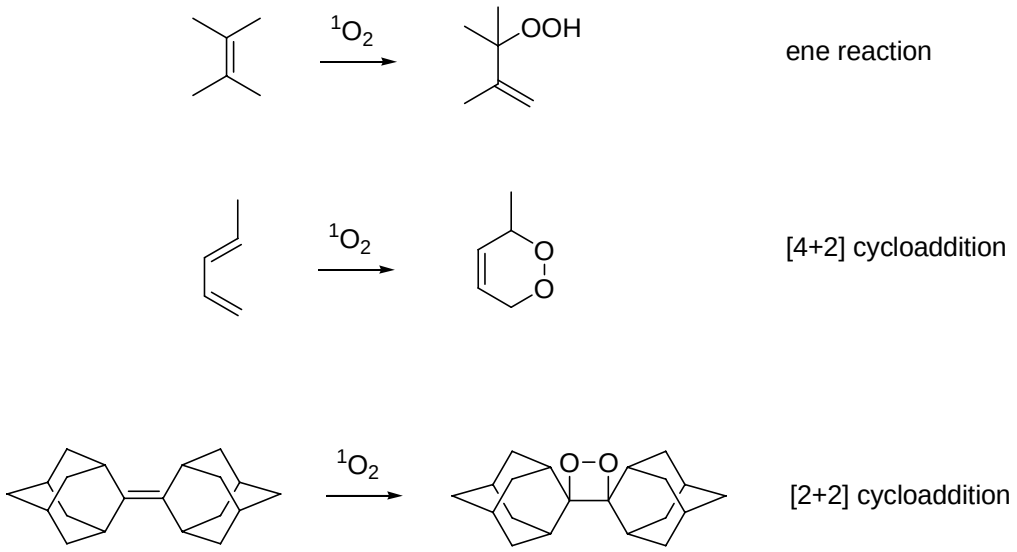
Figure 3.18 – ^{1}H NMR spectrum of **3.81**

This result confirmed that the products of the allylic oxidation of **3.34**, **3.35** and **3.56** are of type **3.76** (**3.77**, **3.78**, **3.79** and **3.80**), where oxidation occurs with concomitant migration of the olefin. It is clear that these enones *cannot* be converted to the desired enedione in the natural product by allylic oxidation.

3.2.7 Cyclopentadiene as an enedione precursor

With the allylic oxidation of olefinic *spiro*-mamakone precursors proving troublesome, alternative strategies for introduction of enedione functional groups were sought.
An alternative method for introduction of an enedione, via singlet oxygen addition to a cyclopentadiene analogue of *spiro*-mamakone A, was examined.

Singlet oxygen is the lowest-energy excited state of molecular oxygen and is commonly generated in a photosensitised reaction via energy transfer from a dye such as Rose Bengal. Its chemistry has been studied for around 50 years and synthetic chemistry has taken advantage of its 3 principal reaction types (**Scheme 3.40**).[96,97]



Scheme 3.40 – The chemistry of singlet oxygen[97]

The main two reactions that singlet oxygen undergoes are the ene reaction and the [4+2] cycloaddition reactions. The ene reaction involves addition to alkenes to generate allylic hydroperoxides with migration of the alkene, which can then be decomposed to synthetically useful allylic alcohols. The [4+2] cycloaddition reaction of singlet oxygen to diene systems generates an endoperoxide which can then be decomposed to an enediol by reduction. The [2+2] cycloaddition reaction of singlet oxygen to form dioxetanes is a more recent addition to singlet oxygen's known chemistry. These have generally been observed in 1,3 dienes where the *cis*- arrangement required for [4+2] cycloaddition is not readily available or those bearing electron-rich substituents.[97]

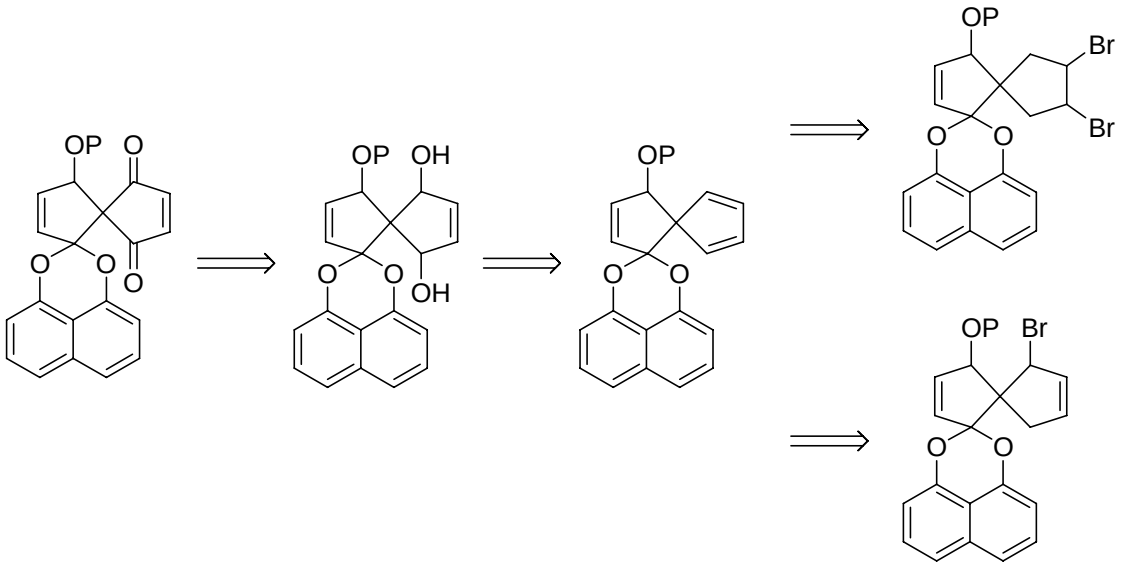
The formation of a 1,4-enediol by [4+2] cycloaddition of singlet oxygen to a diene would, in the case of a *spiro*-disubstituted cyclopentadiene, be expected to be completely regiospecific, introducing oxygen at the two carbons α to the *spiro*- centre. The regiospecific introduction of a 1,4-enediol would generate an excellent precursor to the

1,4-enedione required by *spiro*-mamakone A, by oxidation of the allylic alcohols. This method clearly offered a promising alternative to allylic oxidation for introduction of an enedione, and the generation of a cyclopentadiene derivative of the core *spiro*-mamakone unit was undertaken.

Two approaches were considered for access to a cyclopentadiene derivative of *spiro*-mamakone A. The first approach undertaken was via a bromination, dehydrobromination route (described in **Section 3.2.8**). This method, however, was plagued by problems with regioselectivity. A second, alternative route, took advantage of enones **3.79** and **3.80** via a reduction, elimination method, and allowed access to the key cyclopentadiene derivative (**Section 3.2.9**).

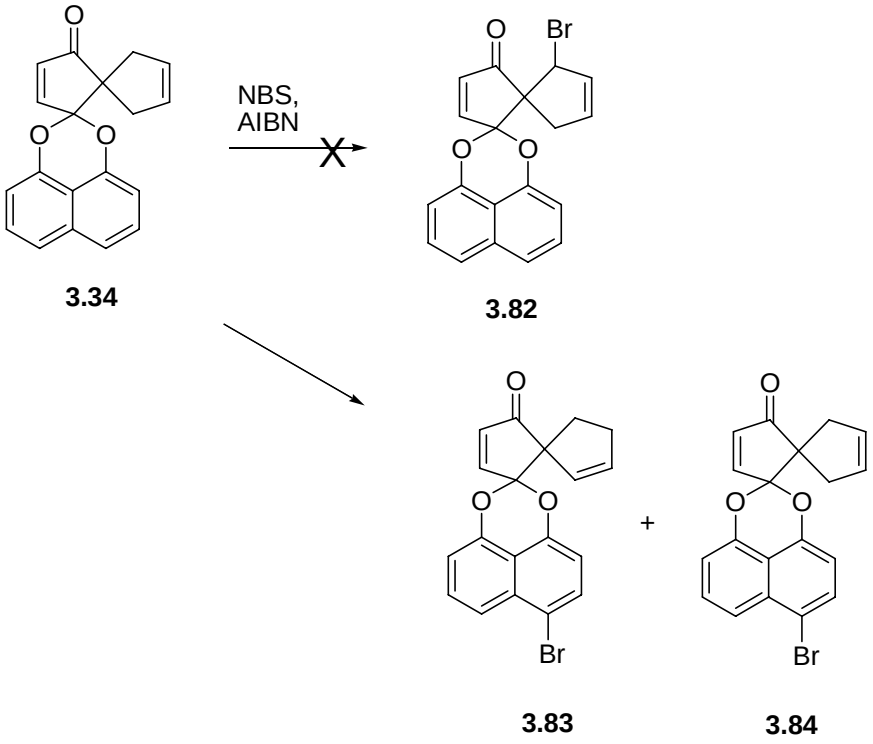
3.2.8 Cyclopentadiene as an enedione precursor – via dehydrobromination

A number of strategies are known for the generation of cyclic dienes which take advantage of dehydrobromination. Classic methods for synthesising 1,3-cyclohexadiene include dehydrobromination of either 3-bromocyclohexene or 1,2-dibromocyclohexane, both of which occur under basic conditions.[98,99] The former is accessed by allylic bromination, and the latter by bromination, of cyclohexene. These strategies could be applied to the synthesis of *spiro*-mamakone by bromination or allylic bromination of the olefin, followed by dehydrobromination (**Scheme 3.41**).



Scheme 3.41 – Alternative strategy based on the oxidation of cyclopentadiene

The allylic bromination of **3.34** was first attempted as a model system (**Scheme 3.42**), using *N*-bromosuccinimide (NBS) and azobisisobutylonitrile (AIBN) as radical initiator. The reaction gave a complex mixture of brominated products, with none of the desired product formed. Three products (^{1}H NMR spectra, **Figure 3.19**) could be isolated by purification on an analytical reverse phase (C_{18}) column. Products **3.83** (an impurity remained) and **3.84** were fully characterised by ^{1}H and 2D NMR spectroscopy (insufficient material collected for ^{13}C NMR spectroscopy) as well as high resolution mass spectrometry. However, the third product was obtained in insufficient quantities for full characterisation.



Scheme 3.42 – Allylic bromination of olefin **3.34**

The pseudomolecular ion ($[M+H]^+$) of both **3.83** (369.0115 Dalton) and **3.84** (369.0131 Dalton) corresponded to the molecular formula $C_{19}H_{13}{}^{79}BrO_3$, showing that a single bromine substitution had occurred. ^{1}H NMR data showed that the distinctive naphthalene proton system (1,2,3-trisubstituted) was not present in both **3.83** and **3.84**, with one of the rings showing only two doublets, suggesting a 1,2,3,4-tetrasubstituted system, and therefore that bromination had occurred on the naphthalene moiety. The low-field aromatic protons (H11/17) were still present in both products, indicating that the bromine substitution had occurred at position H13. Both products showed the expected pair of enone doublets, showing that this central ring was unchanged. ^{1}H NMR data showed that the difference between **3.83** and **3.84** was in the cyclopentene moiety. Olefin **3.83** showed the same distinctive coupling pattern in ^{1}H NMR spectroscopy as had been seen in previous examples where migration of the olefin had occurred (*vide supra*), whereas olefin **3.84** showed the same pattern as that for the starting material. Other 2D NMR data confirmed the assigned structures of **3.83** and **3.84** for these two products.

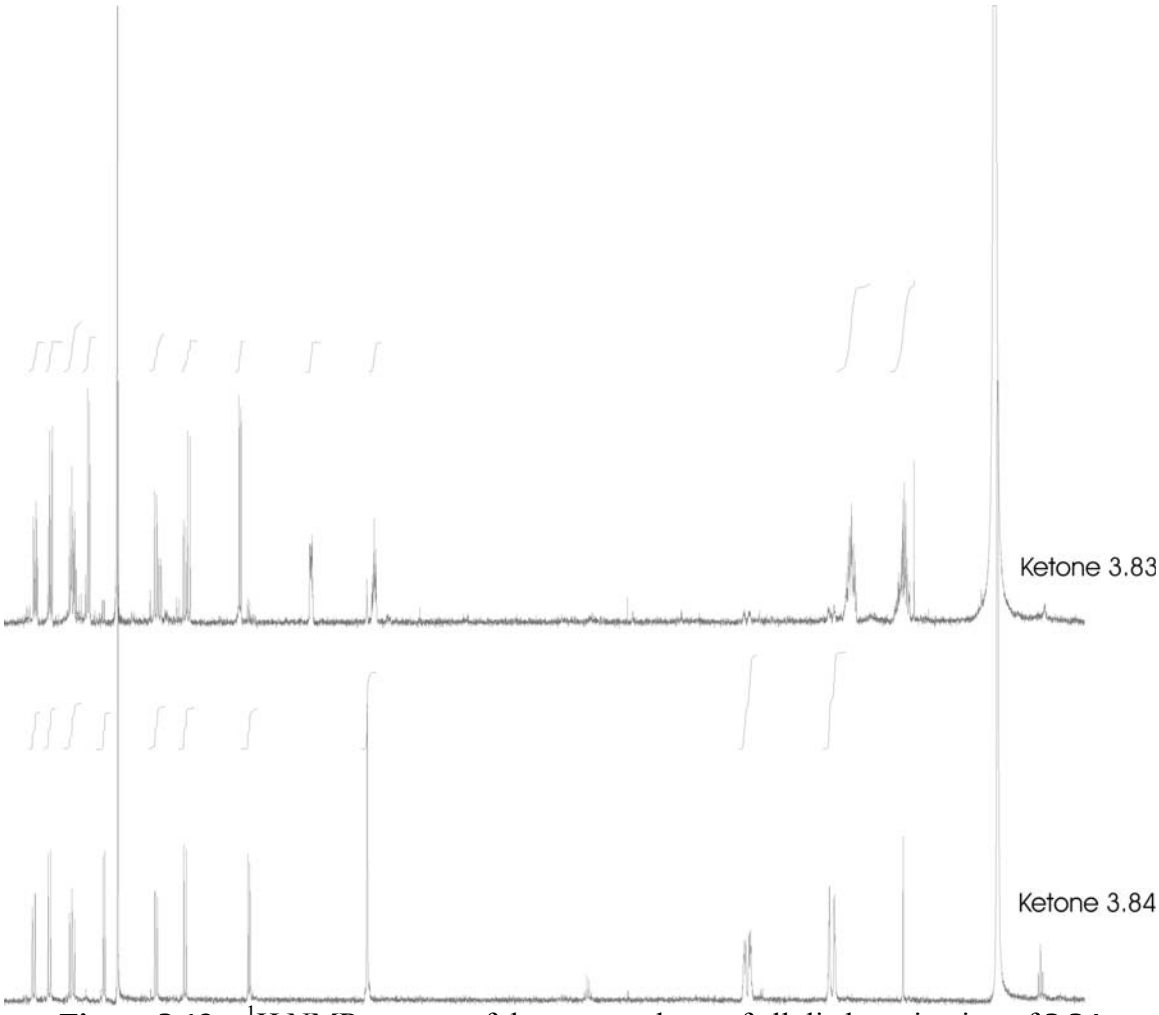
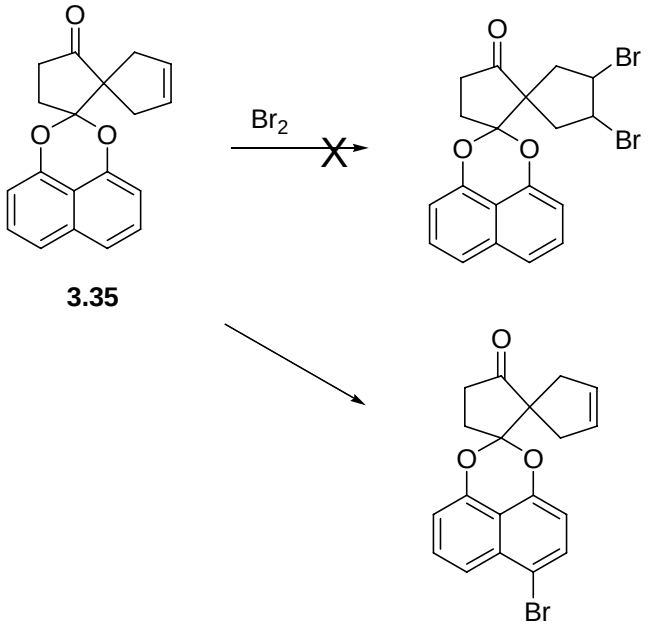


Figure 3.19 – ^{1}H NMR spectra of the two products of allylic bromination of **3.34**

Whilst the allylic bromination of **3.34** had been unsuccessful, the bromination of **3.35** using molecular bromine was also examined (**Scheme 3.43**). Olefin **3.35** was reacted with Br_2 (2.2 equivalents) in carbon tetrachloride. This reaction proceeded more cleanly with some starting material recovered, but only one reaction product obtained. ^{1}H NMR data of this product had the same aromatic pattern as the bromine-substituted naphthalene moiety observed in products **3.83** and **3.84**, suggesting that bromine substitution of H13 had occurred. It also showed that the nonadiene moiety had been unaffected, and the product was tentatively assigned as **3.85**. This compound did not ionise using electrospray ionisation so that no confirmation of the molecular formula could be obtained.

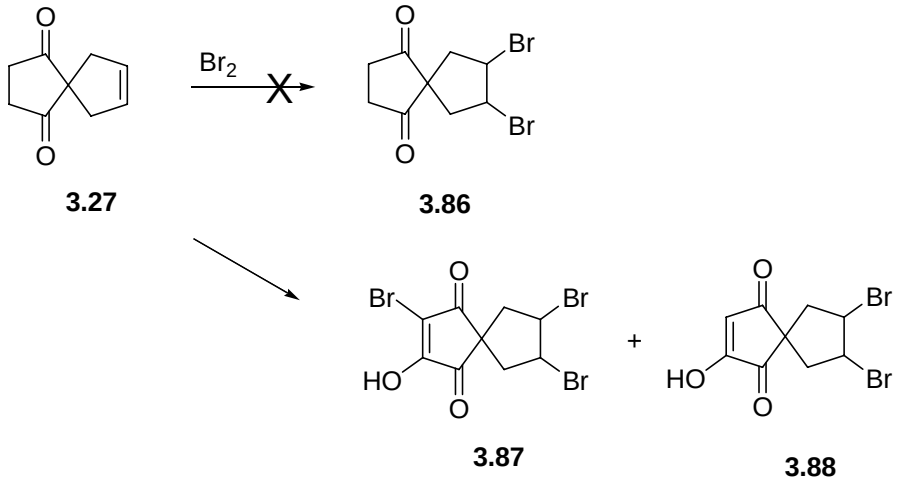


3.35 **3.85**

Scheme 3.43 – Bromination of **3.35**

Under the reaction conditions used above, the electron rich naphthalene moiety was found to dominate the reactivity of the *spiro*-mamakone precursors. The possibility of generating a cyclopentadiene derivative of an earlier intermediate, prior to introduction of the naphthalene moiety was therefore examined.

The bromination of nonadione **3.27** was first examined (**Scheme 3.44**). Bromine (1.2 equivalents) was added to **3.27** in carbon tetrachloride and stirred for 5 hours at room temperature. Despite the rapid decolourisation of the solution, ^{1}H NMR spectroscopy of the crude mixture showed that starting material was largely the only compound present. A further 4.5 equivalents of bromine were added and the mixture stirred for a further 30 minutes, after which time ^{1}H NMR spectroscopy showed that no starting material remained and a mixture of products were present. Purification by chromatography on silica allowed two products to be isolated, each in 38% yield, which were both characterised by ^{1}H and ^{13}C NMR spectroscopy and high resolution mass spectrometry.



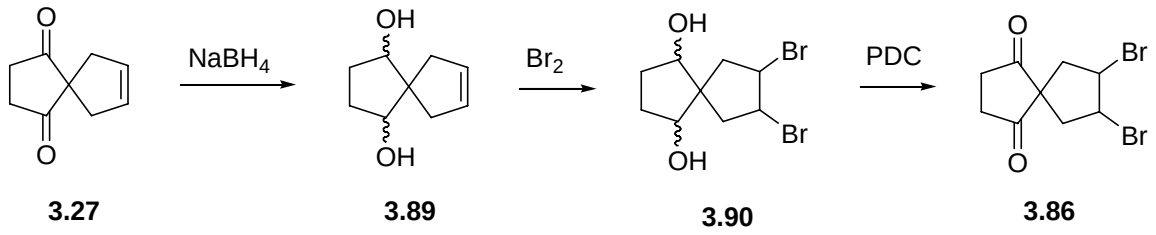
Scheme 3.44 – Bromination of nonadione **3.27**

The mass of the pseudomolecular ion ($[M-H]^-$) for the first product was 398.7882 Dalton, corresponding to a molecular formula of $C_9H_7{}^{79}Br_3O_3$, showing that over-bromination had occurred. ^{1}H NMR data showed three proton environments, integrating equally, at δ_H 4.26, 2.65 and 2.34. If the protons at δ_H 4.26 are taken to correspond to 2 protons, then only a further 4 protons were observed where a further 8 protons would be expected in the desired product. Heavy splitting of the observed protons showed that they were in the same system, suggesting that the olefin had been dibrominated and that the missing protons were those α to the ketones in the left-hand ring. ^{13}C NMR data showed the presence of two carbons at δ_C 194 and 149, suggesting the presence of a substituted enedione moiety. The additional oxygen and bromine given by the molecular formula allowed the product to be assigned as **3.87**.

The psuedomolecular ion ($[M-H]^-$) for the second product was 320.8775 Dalton, corresponding to a molecular formula of $C_9H_8{}^{79}Br_2O_3$. ^{1}H NMR data of this product were very similar to that of the first product, **3.87**, except for an additional singlet at δ_H 7.54 which integrated for 1 proton. This evidence, plus ^{13}C NMR data and the molecular formula led to the assignment of this product as **3.88**. Products **3.87** and **3.88** are postulated to arise from the overbromination of nonadione **3.27**. The one-pot bromination-dehydrobromination of ketones by Br_2 to give enones has been previously

described and is presumed to occur in this case.[100] How the substitution of this enone occurs to yield **3.87** and **3.88** is less clear, however.

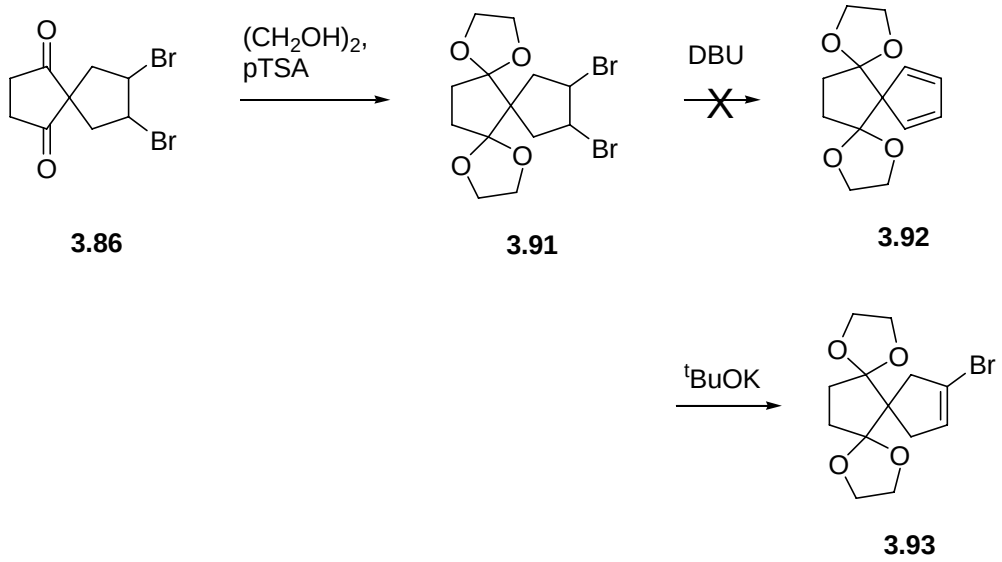
The bromination of nonadione **3.27** showed that the reactivity of the α-keto methylenes towards bromine caused side-reactions to occur. In an attempt to resolve this problem the reduction of both ketones in **3.27** was carried out using sodium borohydride in methanol to give the diol **3.89** in approximately quantitative yield (**Scheme 3.45**). Diol **3.89** was obtained as a mixture of stereoisomers, but since after bromination the ketones would be reformed, no attempt was made to separate these isomers. The bromination of diol **3.89** using bromine now proceeded cleanly to give the dibrominated nonadiol **3.90**, in approximately quantitative yield. Reoxidation of the alcohols could be carried out using 6 equivalents of pyridinium dichromate to give dibrominated nonadione **3.86** in 50% yield.



3.27 **3.89** **3.90** **3.86**

Scheme 3.45 – Alternative route to dibrominated nonadione **3.86**

In order to prevent base-catalysed side-reactions during dehydrobromination, protection of the ketones of nonadione **3.86** was undertaken (**Scheme 3.46**). Refluxing **3.86** under Dean-Stark conditions with an excess of ethylene glycol readily yielded the doubly protected ketal derivative **3.91** in approximately quantitative yield.



3.86 **3.91** **3.92** **3.93**

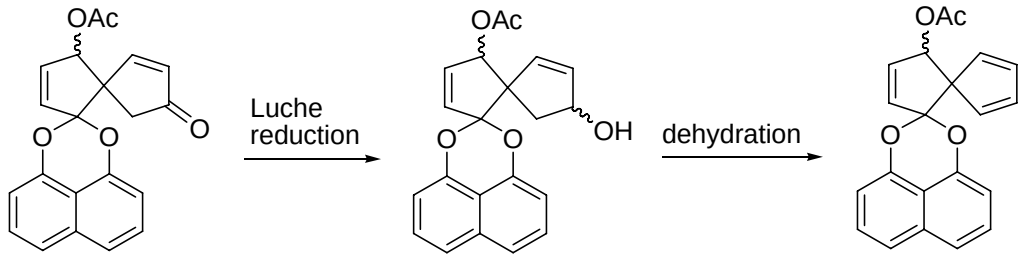
Scheme 3.46 – Attempted dehydrobromination of protected nonadione **3.91**

This was then submitted to base-catalysed dehydrobromination, with DBU. After heating the reaction in acetonitrile at 70ºC for 4 days or in DMF for 2 days, only starting material was recovered. These conditions, using a mild, hindered base, promote an E_1 elimination mechanism and are standard for dehydrobromination. The lack of reaction showed that an E_1 elimination was not favoured in this system. The use of a strong unhindered base, which might be expected to favour an E_2 mechanism, was briefly examined to see if it promoted dehydrobromination. Dibromide **3.91** was reacted with an excess of potassium ^{t}butoxide. This caused mono-dehydrobromination to occur, however because the most accessible proton was removed leading to product **3.93**, the desired cyclopentadiene could not be formed. Product **3.93** was fully characterised by ^{1}H, 2D and ^{13}C NMR spectroscopy and high resolution mass spectrometry.

The possibility of attempting an allylic bromination of **3.89** to generate a 3-bromoalkene derivative of **3.27** could have been next attempted. However, the generation of enones **3.79** and **3.80** (*vide supra*) opened up the opportunity to generate a cyclopentadiene derivative of the *spiro*-mamakone core structure by a different method and this route was successfully examined instead (**Section 3.2.8**).

3.2.9 Cyclopentadiene as an enedione precursor – via allylic alcohol elimination

A route to a cyclopentadiene from enones **3.79**/**3.80** generated during earlier studies (**Section 3.2.6**) can be envisioned (**Scheme 3.47**) via reduction to an allyl alcohol and dehydration. Indeed the first of these steps had already been achieved (*vide supra*) and the dehydration of the alcohol previously observed by mass spectrometry. This route was therefore examined.



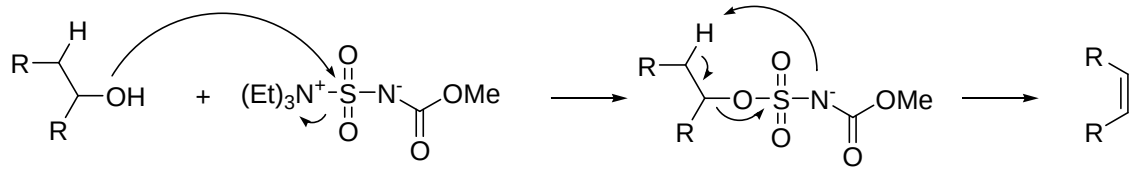
3.79/3.80 **3.94** **3.95**

Scheme 3.47 – Preparation of cyclopentadiene **3.95** from enones **3.79/3.80**

Whilst the Luche reduction of enone **3.79** had been achieved previously using a diastereomerically pure sample of **3.79**, all stereochemical entities of **3.94** would be resolved on dehydration to give a single enantiomeric pair of **3.95**. This allowed a mixture of enones **3.79** and **3.80** to be employed in this route. Luche reduction gave a complex mixture of stereoisomers of allyl alcohol **3.94** (including **3.81**), as anticipated.

The dehydration of the allyl alcohol **3.94** was next undertaken. A variety of dehydrating conditions are known to effect the dehydration of cyclic allylic alcohols to generate dienes. The most common involve the addition of catalytic quantities of acid to promote the loss of water. The possible sensitivity of the spiroketal moiety to such conditions make these unattractive, and non-acidic conditions were sought. Multi-step methods involving activation of the alcohol as a sulfonate ester and followed by elimination with base offered an alternative route. However, a promising reagent which has been used to yield cyclopentadienes from cyclic allylic alcohols[101] was the inner salt of (carboxysulfamoyl)triethylammonium hydroxide methyl ester, **3.96**, also known as

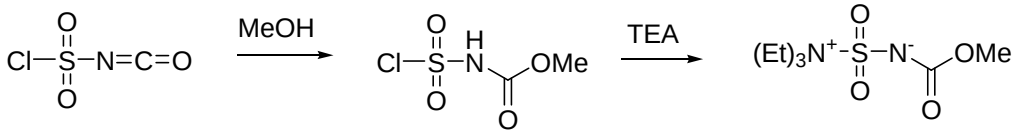
Burgess reagent.[102,103] It effectively combines the activation of the alcohol as a sulfonate ester and base-catalysed elimination in one step, as the direct result of being an inner salt. The accepted mechanism is described in **Scheme 3.48**. It is a mild, non-acidic and efficient dehydrating agent. Despite being an inner salt, it is readily soluble in most organic solvents and effects pyrolytic dehydration at relatively low temperatures (generally less then 100ºC).



3.96

Scheme 3.48 – Mechanism of Burgess reagent mediated alcohol dehydration

The preparation of Burgess reagent was carried out in two steps from readily available chlorosulfonyl isocyanate (**Scheme 3.49**).[104] The first step proceeded in 81% yield, and the second in 63% yield. Both products were obtained as white solids whose melting points were in good agreement with literature values.



3.96

Scheme 3.49 – Preparation of Burgess reagent (**3.96**)

The dehydration of **3.94** was successfully carried out by warming (50ºC) with 2 to 3 equivalents of Burgess reagent in benzene overnight. Whilst crude ^{1}H NMR data suggested high conversion, relatively low isolated yields of around 30% were obtained. The product was fully characterised by ^{1}H (**Figure 3.20**) 2D and ^{13}C NMR spectroscopy as well as high resolution mass spectrometry. All data confirmed the formation of cyclopentadiene derivative **3.95**.

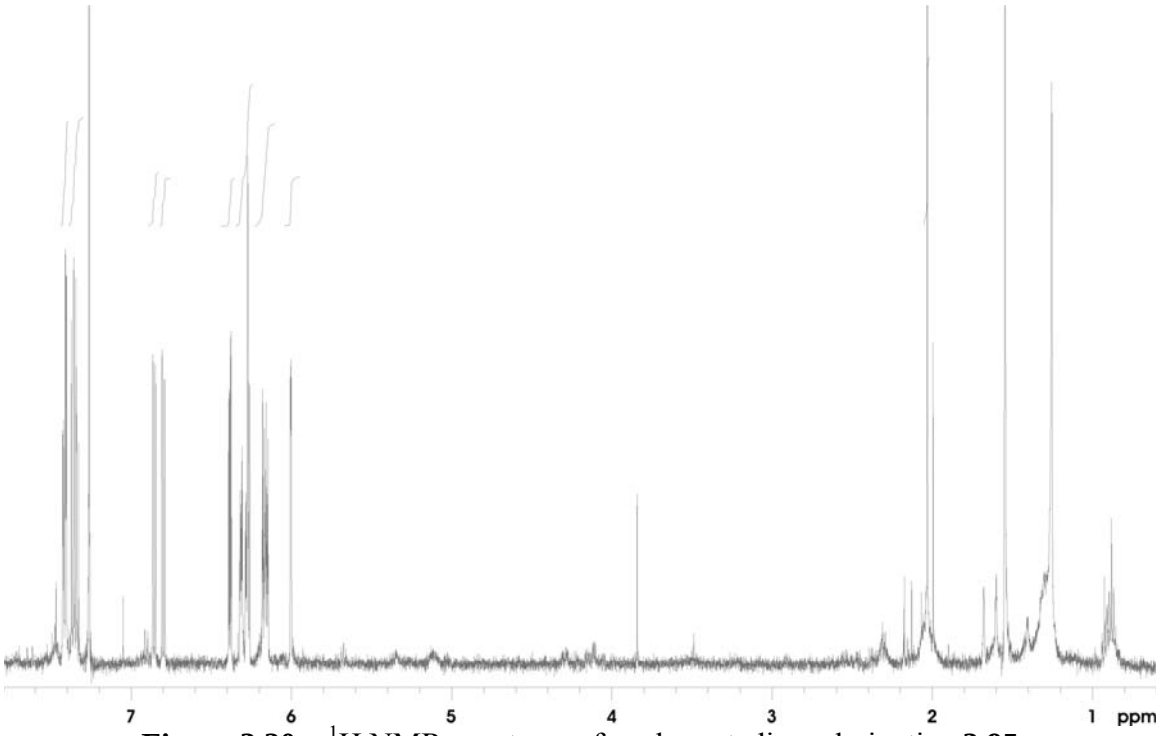


Figure 3.20 – ^{1}H NMR spectrum of cyclopentadiene derivative **3.95**

3.2.10 Singlet oxygen addition to a cyclopentadiene derivative

With cyclopentadiene derivative **3.95** in hand, singlet oxygen oxidation to generate a 1,4-enediol was attempted. Examination of the literature for singlet oxygen addition to similar precursors allowed the choice of Rose Bengal as photosensitiser, with methanol or methanol/DCM mixtures as solvent using a tungsten lamp as the source of visible light.[105,106,107] Where the desired product was an enediol several groups included thiourea as a reducing agent to reduce *in situ* the initially formed endoperoxide. This avoids the need to isolate the sensitive endoperoxide before reduction. These conditions were employed in the oxidation of cyclopentadiene **3.95**. The reaction was placed under a balloon of oxygen with vigorous stirring to ensure good availability of oxygen in the solution. The reaction was initially carried out in an ice-bath, however HPLC analysis of the crude reaction mixture after 2 hours showed no reaction was occurring, so it was warmed to room temperature and the reaction continued overnight. HPLC analysis (**Figure 3.21**) now showed that almost all starting material had been consumed and two

new major products had been formed. These two products were both significantly more polar than the starting material and eluted very closely, with a minor product eluting between them. Since the reaction had been carried out on a very small scale, the products were purified by chromatography on HPLC using an analytical C_{18} column. Whilst the two major and a minor impurities were not well resolved under the elution conditions used above for analysis, an initial purification was carried out using these conditions with both major products collected together. ^{1}H NMR spectroscopy of this mixture showed the presence of two related compounds, but further purification was undertaken before full characterisation. The mixture was purified again by HPLC on an analytical C_{18} column using different elution conditions to give the two products as amorphous white solids.

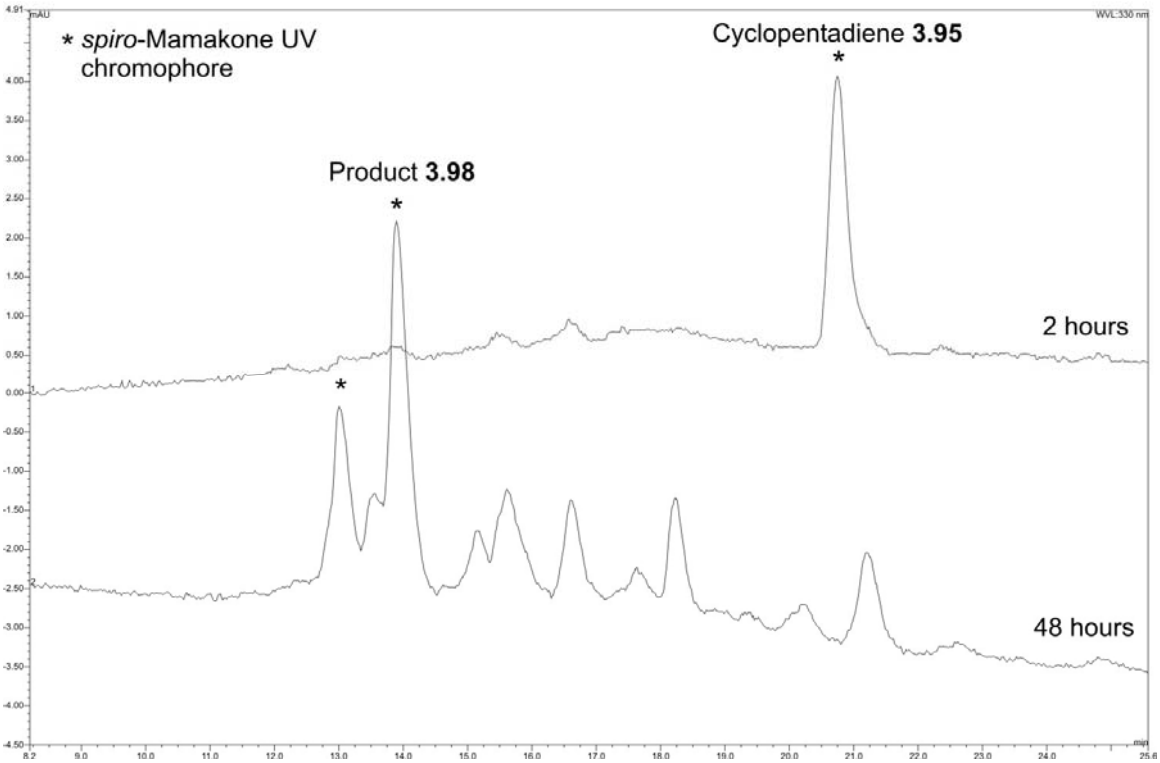


Figure 3.21 – HPLC analysis of singlet oxygen oxidation of cyclopentadiene **3.95**

The major of the two products (the second eluted) was characterised by ^{1}H (**Figure 3.22**) and 2D NMR spectroscopy (COSY and HSQC, and partial HMBC data were obtained). ^{1}H NMR spectroscopy showed that the naphthalene moiety and the acetyl protected

allylic alcohol were both present and broadly unchanged. Four other protons were observed at δ_H 6.16, 5.88, 5.48 and 3.86. HSQC NMR data showed that the two down-field protons were attached to carbons at δ_C 132.2 and 133.6 respectively. The two upfield protons were attached to carbons at δ_C 86.0 and 65.0. COSY correlations could be seen between the two down-field protons and the two up-field protons, but no correlation was observed between the two pairs (see **Figure 3.23**).

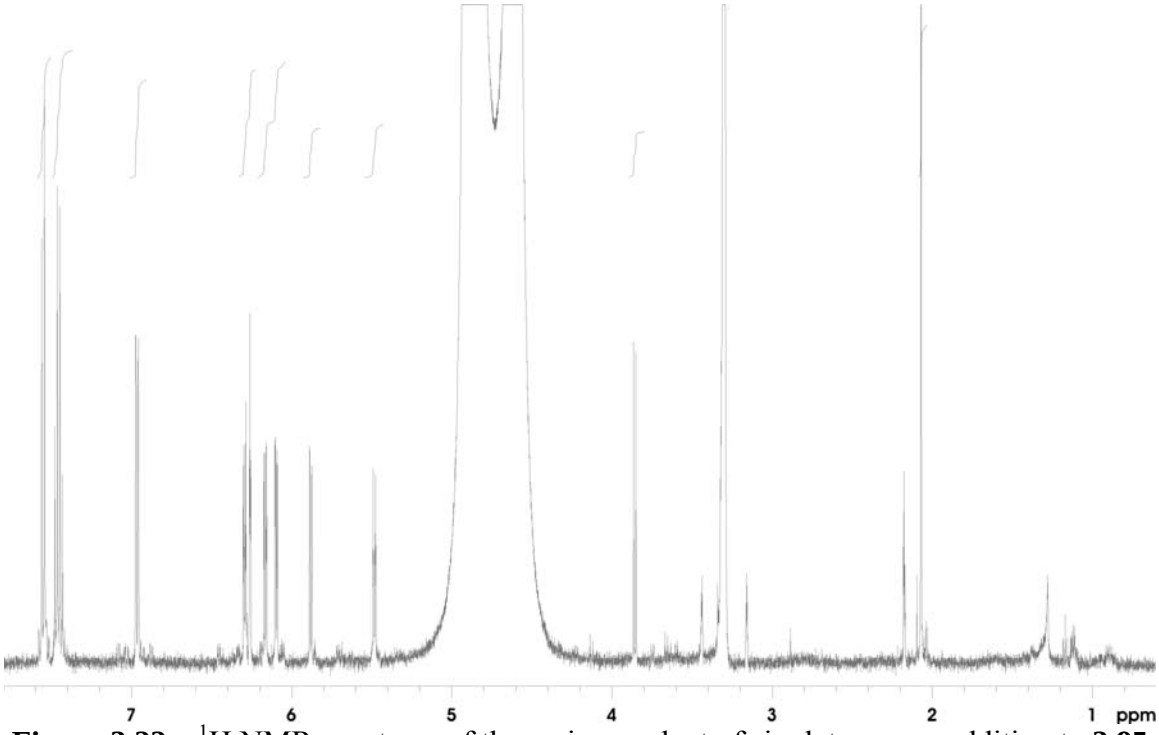
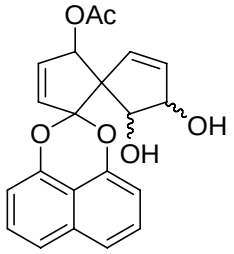


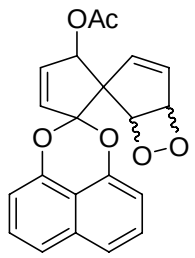
Figure 3.22 – ^{1}H NMR spectrum of the major product of singlet oxygen addition to **3.95**

These data suggested that the anticipated 1,4-enediol had not been formed. This product would be anticipated to have two allylic alcohol protons and two olefinic protons with a high degree of symmetry. Apart from the lack of correlation between the two pairs of protons, the data suggested that the product formed could be a 1,2-enediol of type **3.97**. The lack of an observable COSY correlation between the two pairs is likely to be due to a dihedral angle of around 90º, which leads to very small coupling constants, making COSY correlations weak. Due to a lack of material, no useful HMBC data could be obtained to confirm this proposed connectivity.

Whilst two olefinic protons and one allylic alcohol proton (δ_H 5.48) were present, the chemical shift of the proton at δ_H 3.86 was more indicative of an aliphatic alcohol. The chemical shifts (δ_H 5.48, δ_C 86.0) of the allylic alcohol were somewhat higher than might be expected. The product did not ionise under ESIMS or CIMS conditions, however under high resolution EIMS the pseudomolecular ion observed was 364.0935 Dalton, corresponding to a molecular formula of $C_{21}H_{16}O_6$. This molecular formula has two less protons than enediol **3.97**, and suggests that reduction has not occurred, and that the product of the reaction may instead be **3.98**. This assignment is supported by the observation of two low resolution EIMS fragmentation products observed; *m*/*z* = 332 (loss of O_2) and *m*/*z* = 290 (loss of O_2 and $COCH_2$). In addition, this proposed structure may account for the unusually high chemical shifts of the allylic alcohol. Whilst 2D NOESY NMR data were obtained, no correlations were observed which could help establish the stereochemistry of the product.



3.97



3.98

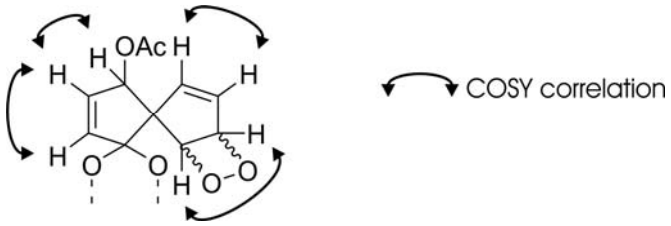


Figure 3.23 – observed COSY correlations for **3.98**

The minor product (first eluted) of the singlet oxygen oxidation of **3.95** was obtained in smaller quantities which were insufficient to fully characterise this compound. ^{1}H (**Figure 3.24**), COSY and partial HSQC NMR data were obtained, however, and indicated that this product was very similar to the major product. ^{1}H NMR data showed a similar pattern to the major product with small differences in chemical shift (one proton

was obscured by the residual solvent peak, but was observed in a COSY NMR experiment). Two possible structures can be readily postulated, either this product may be a diastereoisomer of the major product, or it is the reduced form of the product, **3.97**. However, the similarity in the retention time (and therefore polarity) of these two products would suggest that it is not the diol **3.97**, since this would be expected to be markedly more polar than **3.98**. It is therefore postulated that this product represents a diastereoisomer of the major product, although no data were obtained to determine either products' relative or absolute stereochemistry. The difference in stereochemistry between the two products might arise either from addition of oxygen from the top or bottom face of the cyclopentadiene (relative to the naphthalene moiety) or from its addition on the same or opposite side to the protected allyl alcohol on the central ring. This compound did not ionise in ESIMS, so the proposal that it is a stereoisomer of **3.98** could not be confirmed by comparison of the molecular weight.

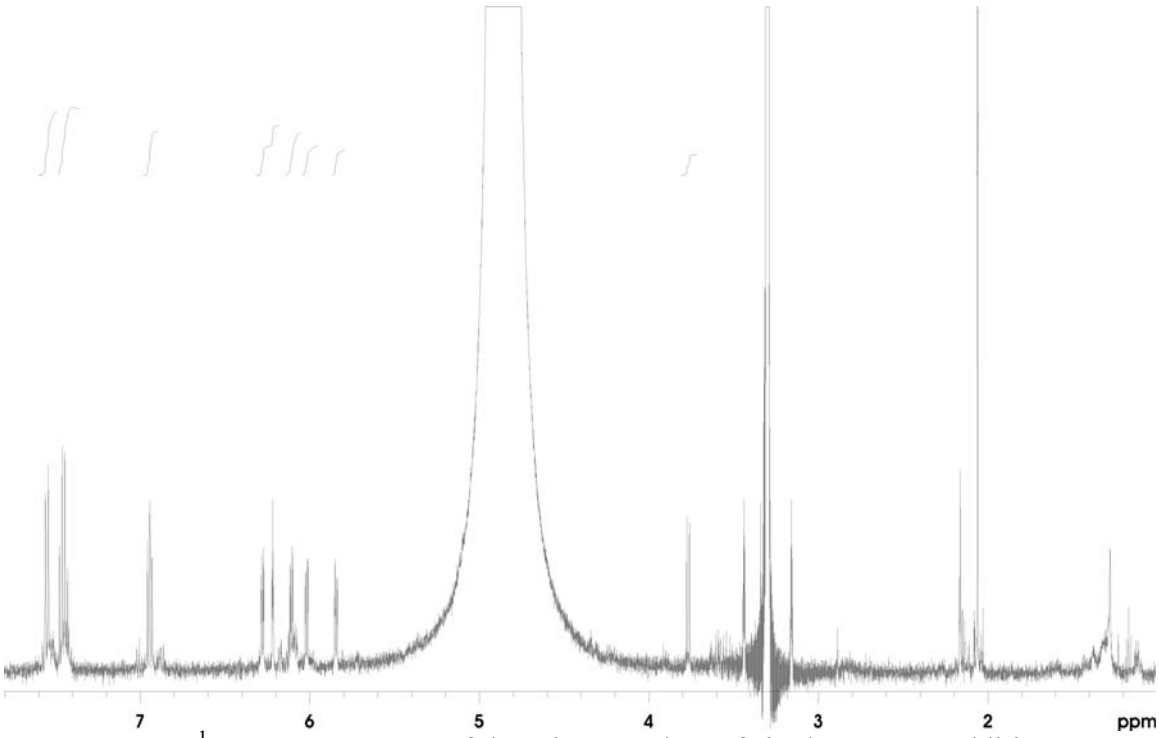


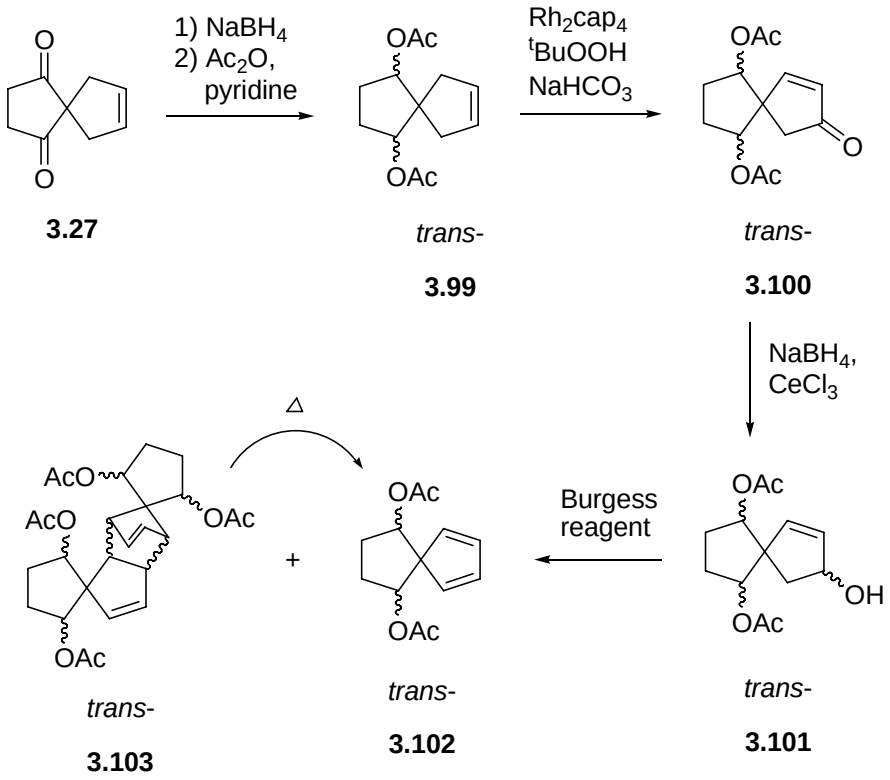
Figure 3.24 – ^{1}H NMR spectrum of the minor product of singlet oxygen addition to **3.95**

The proposed products of the reaction of singlet oxygen with **3.95** are highly unexpected. The cycloaddition of singlet oxygen to dienes should reliably proceed regiospecifically at the 1,4- position to generate a 1,4-enediol. The generation of a 1,2-enediol might, at first sight, seem to have arisen from a [2+2]-cycloaddition. However, as mentioned above, those literature examples of [2+2]-cycloaddition to dienes occurs where the *cis*- position is inaccessible or where electron-rich substituents are present on the diene. Neither of these scenarios are true in the present case, indeed **3.95** seems an ideal substrate for a [4+2]-cycloaddition. Re-examination of the literature brought to light the possibility that [4+2]-cycloaddition was occurring, but that rearrangement of the endoperoxide to a dioxetane may then occur.[108,109,110] This is therefore proposed as the most likely mechanism by which the diastereoisomeric products of **3.98** arise from the singlet oxygen oxidation of **3.95**.

In order to generate the desired 1,4-enediol precursor to *spiro*-mamakone A, the rearrangement of the endoperoxide to a dioxetane would need to be suppressed such that the reduction of the endoperoxide can occur. The rearrangement of an endoperoxide is considered to be a thermal rearrangement, such that at low temperatures the endoperoxide is more stable.[110] The singlet oxygen oxidation of **3.95** was therefore attempted at -78ºC and monitored by HPLC. After 8 hours only the starting diene was present and no photooxidation products were in evidence. The reaction was therefore reattempted at -45ºC and, again, monitored by HPLC. After 16 hours, largely starting material remained, however two new peaks were observed by HPLC, with the characteristic naphthalenediol UV spectrum. These two peaks were purified by chromatography on the C_{18} analytical column. Unfortunately both samples were shown to be mixtures of compounds, and were present in too small quantities for further purification and characterisation of the products. It was clear that whilst at low temperature photooxidation of **3.95** would proceed, it did not proceed at an appreciable rate and led to complex mixtures of products.

Since **3.95** was a very advanced intermediate, only minimal quantities were available. The formation of the acetal was the principal 'bottleneck' in this synthetic route, with yields generally of only around 10 to 15%. None of the subsequent synthetic steps

reliably proceeded at quantitative yields, such that further mass was lost at each stage. The total quantity of **3.95** that was obtained was only around 3-4 mg. This severely limited the possibility of variation of the reaction parameters of the singlet oxygen addition to improve the reaction outcome. The preparation of a model compound which could be prepared on a moderately large scale to test the parameters of the singlet oxygen oxidation was therefore undertaken. An ideal model approximates the final system closely whilst being readily available on a reasonably large scale. Cyclopentadiene **3.102** seemed an appropriate model and its synthesis was undertaken (**Scheme 3.50**). This protected diol cyclopentadiene was chosen over a dione cyclopentadiene since attempts to achieve the allylic oxidation of **3.27** yielded only starting material, similarly to the allylic oxidation of acyclic olefin **3.26** discussed earlier (**Section 3.2.5**). Since reduction and protection of the ketones had allowed successful allylic oxidation in that case, it was also applied here, successfully.



Scheme 3.50 – Preparation of a model cyclopentadiene system

The double reduction of dione **3.27** (200 mg) was readily achieved using an excess of sodium borohydride in methanol to give the separable *cis*- and *trans*- diastereoisomers of diol **3.89** with an isolated yield of 43% for the *trans*- isomer. For ease of analysis of ^{1}H NMR spectroscopy at later stages in the synthesis only the *trans*- diastereoisomer was carried through the synthesis. The diol was protected as an acetate under standard conditions (acetic anhydride and pyridine) to give **3.99** in 75% yield. Allylic oxidation of protected diol **3.99** gave enone **3.100** in 79% yield. Reduction of the enone to an allylic alcohol was achieved under Luche reduction conditions, as previously, to give the product as a mixture of stereoisomers in 88% isolated yield. The allylic alcohol, **3.101**, was subjected to dehydration conditions using Burgess reagent (50ºC, 7 hours). Whilst the cyclopentadiene product, **3.102**, was obtained from this reaction, it was only recovered in an approximate yield of 11% and was still contaminated by impurities. A second fraction also obtained from this reaction had a complicated ^{1}H NMR spectrum. This product was postulated to be a mixture of Diels-Alder products (**3.103**) arising from the dimerisation of the cyclopentadiene **3.102**. This postulate was supported by mass spectral analysis, which showed the pseudomolecular ion of the cyclopentadiene monomer, **3.102**. The observance of the pseudomolecular ion of a monomer is common in the mass spectral analysis of Diels-Alder derived dimers due to retro-Diels-Alder processes under the harsh ionisation conditions employed. This product was therefore subjected to conditions aimed at achieving a retro-Diels-Alder reaction – refluxing in a highly dilute solution of benzene for 3 days. After this time, ^{1}H NMR spectroscopic analysis of the crude mixture showed the presence of the monomer and no dimer remained. Disappointingly, however, only a further 6% of the monomer was isolated, although this sample was pure.

The disappointing yields of the dehydration step during the synthesis of this cyclopentadiene model system resulted in an overall yield of only 4 mg of the pure final cyclopentadiene, **3.102**, from 88 mg of starting *trans*-diol **3.89** (or 200 mg dione **3.27**). As a result this model system would not allow an extensive examination of the reaction conditions of singlet oxygen oxidation to these *spiro*-cyclopentadiene systems. Nevertheless, the oxidation was attempted under the same conditions as employed for the

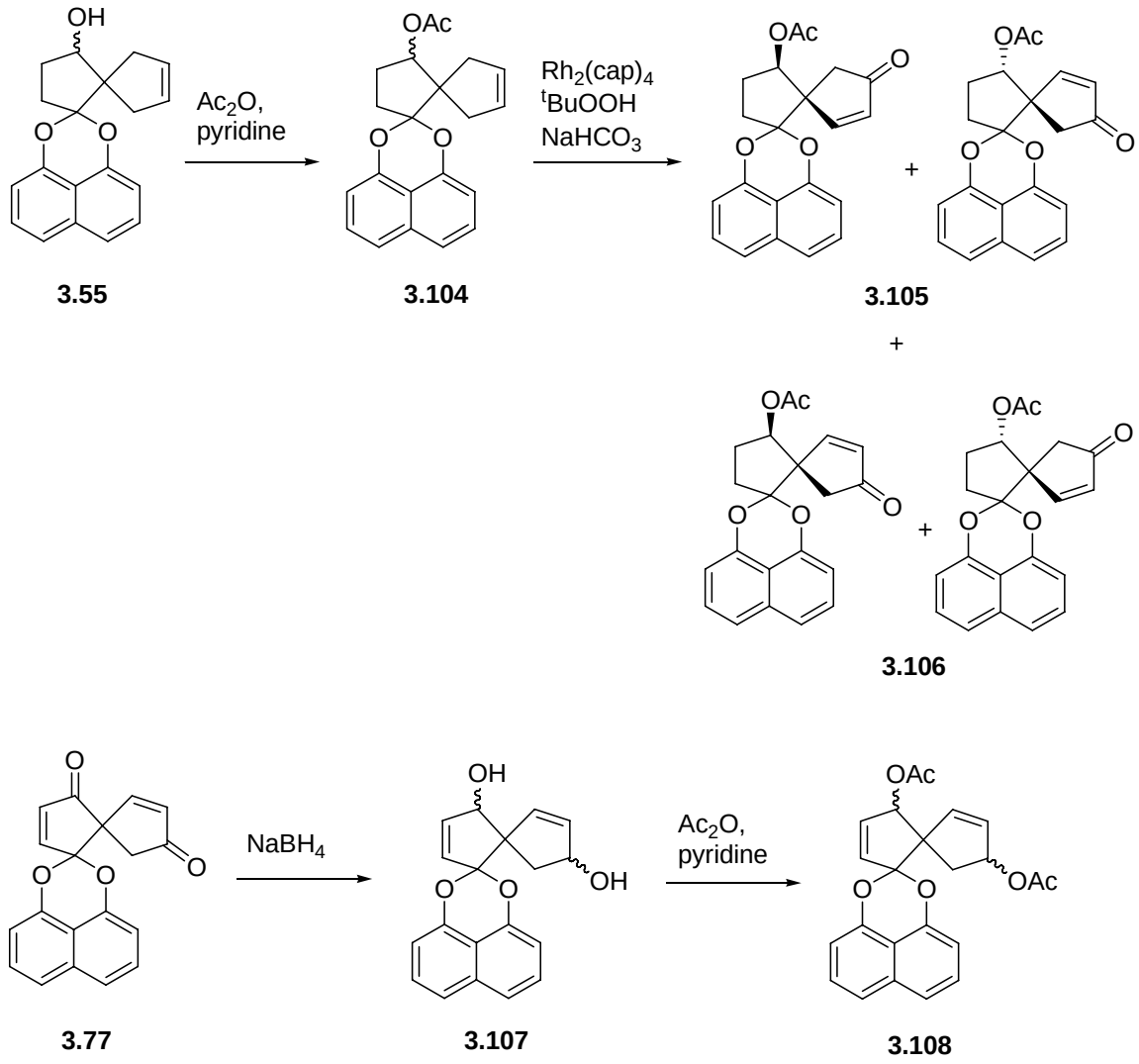
oxidation of **3.95**, above, on around one quarter of the mass of **3.102**. The ^{1}H NMR spectrum of the crude sample showed that a complex mixture of products had been formed. On this scale, the analysis of a complex mixture of products would have been untenable, so the remaining **3.102** was also submitted to the same conditions employed for oxidation of **3.95**. Again a complex mixture was obtained, although a major product was the enone **3.100**. A plausible mechanism for the regeneration of enone **3.100** is via a 1,2-diol. The initial generation of a dioxetane, as seen in previous examples, may be reduced *in situ* to a 1,2-diol, which can then eliminate water to generate an enol, which would tautomerise to enone **3.100**. The recovery of this product therefore suggests, indirectly, that the dioxetane intermediate was generated from the endo-peroxide. No other products could be isolated in sufficient quantity to allow characterisation or structure elucidation.

The model system had therefore not generated any useful information on how to alter the conditions of the singlet oxygen cycloaddition of **3.95**. The insufficiency of material of **3.95** and time constraints imposed on this project forced a reluctant abandonment of further synthetic attempts towards *spiro*-mamakone A.

3.3 Biological activity of *spiro*-mamakone synthetic analogues and analysis of structure-activity relationships

A number of intermediates were obtained during the course of the above synthetic efforts to prepare *spiro*-mamakone A. Many of these closely resemble in structure the natural product, which is known to be potently bioactive in a P388 leukaemia assay (IC_{50} of 0.33 μM). The other natural products obtained in the *spiro*-mamakone series also displayed cytotoxicity (IC_{50} values of 1.39 μM, 0.95 μM and 3.76 μM respectively for *spiro*-mamakone B, C and D). It was therefore valuable to examine the bioactivity of the intermediates generated in this synthetic work, in order to allow a structure-activity profile to be developed. In addition to cytotoxic activity (as evidenced by the P388 leukaemia assay), the natural products also displayed antimicrobial activity, however only the cytotoxicity of the synthetic analogues was tested (**Figure 3.25**).

In an effort to expand the range of analogues tested, a small number of simple reactions were carried out on some of the intermediate analogues described above (**Scheme 3.51**). Alcohol **3.55** was protected as an acetate, to give **3.104**, a close analogue of **3.56**. Like **3.56**, **3.104** was also subjected to allylic oxidation to give two diastereomeric enones (racemic mixture of enantiomers), **3.105** and **3.106**. Protected alcohol **3.104** and enones **3.105** and **3.106** were fully characterised by ^{1}H, 2D and ^{13}C NMR spectroscopy and high resolution mass spectrometry. A correlation between the acetate proton (H4) and one the methylene protons adjacent to the enone in a 2D NOESY experiment of **3.106** (not present in compound **3.105**) allowed these two enones to be distinguished. The Luche double reduction of **3.77** gave **3.107** as a mixture of stereoisomers which could not be separated. The diols of **3.107** were protected as acetates using classic conditions to give **3.108** as a mixture of stereoisomers. Because of the complex stereochemical mixtures of **3.107** and **3.108**, only ^{1}H and high resolution mass spectral data were used to elucidate their structures.



Scheme 3.51 – Preparation of additional analogues of *spiro*-mamakone A for biological testing

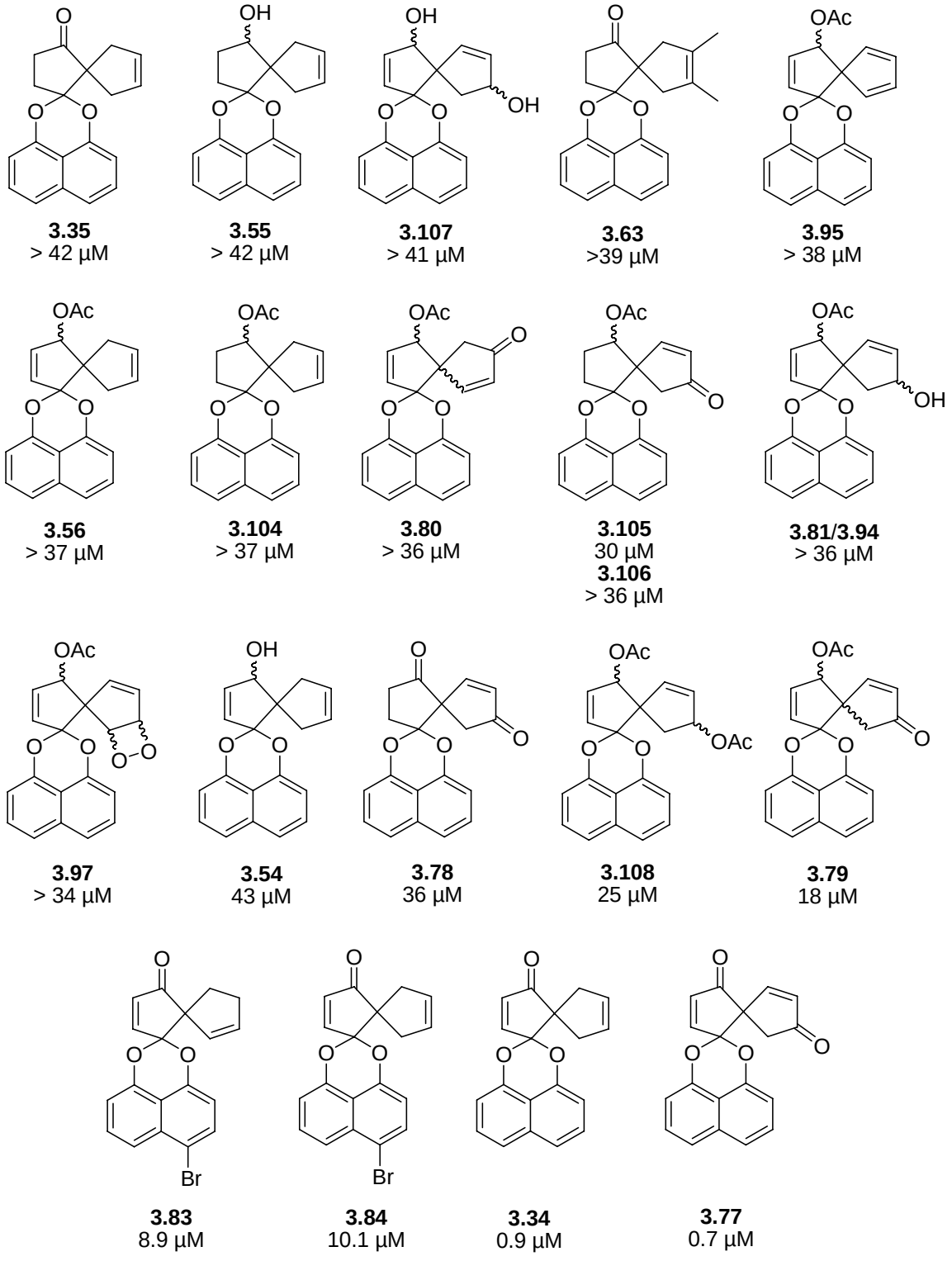


Figure 3.25 – Results of biological testing (P388 murine leukaemia cell line assay) on *spiro*-mamakone analogues. (IC_{50} values shown)

More than half of the analogues tested did not show any activity in the concentration range tested, ie they did not show any cytotoxicity below concentrations of around 30-40 μM. No further tests were carried out at higher concentrations to determine the concentration at which they would show cytotoxicity. All of these analogues have the same basic carbon skeleton as the natural *spiro*-mamakone series, although they vary quite widely in the range and placement of functional groups on the skeleton. Amongst these, **3.107** is interesting in that it contains the same central ring allylic alcohol motif, but has a second allylic alcohol in the right-hand ring rather than an enedione. This clearly demonstrates the importance of the enedione motif in the cytotoxic effect of the natural products. The very weak activity of **3.54** also supports this conclusion.

A small number of the analogues tested displayed potent cytotoxicity, in particular **3.34** and **3.77**. These show activities comparable to the natural product series (*spiro*-mamakone A displayed an IC_{50} of 0.33 μM). In addition, several compounds displayed moderate cytotoxicity of between 8.9 and 43 μM. These compounds also span a range of functionality making general conclusions difficult to draw. Many of the compounds which display strong to moderate cytotoxicity include one or more enone moieties at various positions. Enones are strongly electrophilic, readily undergoing 1,4-additions with nucleophiles. It may be this reactivity which is dominating the cytotoxic effect by interacting with nucleophiles such as cysteine residues in proteins. The absence of an enone means that this sort of reactivity is not possible in the natural product series. The enedione moiety present in the natural series is a significantly less polarised functional group and is therefore much less prone to undergo 1,4-additions with nucleophiles. This suggests that the cytotoxicity observed with the enone-containing analogues may derive their cytotoxicity via a different mechanism to the natural series. However, a number (**3.80** and **3.106**) of biologically inactive analogues also contain enone moieties, so the assumption that enone containing analogues are acting via 1,4-additions to nucleophiles may be an over-simplification.

Some stereochemical dependence can be observed in the biological activities of the synthetic analogues. For example in the case of stereoisomeric enones **3.79** and **3.80**, one shows moderate bioactivity whilst the other is inactive.

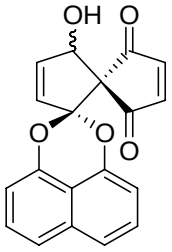
Overall, these results demonstrate that the *spiro*-mamakone series represent highly refined entities with respect to their biological activity. Many quite closely related analogues were synthesised which showed no activity, or greatly reduced activity compared to the natural products. A small number of synthetic analogues did display biological activity analogous to the natural products. These all contained the reactive enone motif, which may impart biological activity by different mechanisms to the natural product.

These results provide useful information on the structure-activity relationship profile of the *spiro*-mamakone series. Further work which could expand upon the above results would be testing of both the natural products and synthetic analogues against more specific biological assays, to elucidate the mechanism of action of these compounds.

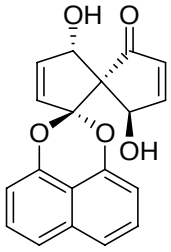
3.4 Future Work

The work presented in this thesis towards the synthesis of *spiro*-mamakone natural products provides a solid basis for further synthetic investigations, with the obvious end-point of completing the total synthesis of these natural products. It may be possible, with further experimentation, to alter the regioselectivity of the singlet oxygen addition to give the crucial enedione moiety required of the natural products. Improving the overall yield of the synthetic route to the cyclopentadiene precursor of this oxidation would greatly facilitate this goal. Alternatively, entirely different synthetic routes may be required to access the *spiro*-mamakones.

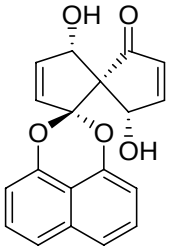
Synthetic routes which allow access to all of the *spiro*-mamakones would be beneficial. Diastereoisomers *spiro*-mamakones B and C are closely related to *spiro*-mamakone A; the only difference being the reduction of one ketone of the enedione moiety to an alcohol. Preparation of *spiro*-mamakones B and C is therefore likely to be readily achievable by classic reductive methods (eg the Luche reduction) from *spiro*-mamakone A, although regio- and stereochemical control is likely to be limited. *spiro*-Mamakones D and E are not so closely related and include additional oxygenation of the right-hand ring. The dioxetane formation observed during singlet oxygen addition in this thesis may offer opportunities to access the *spiro*-mamakone D and E oxygenation pattern.



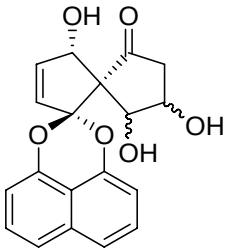
3.1
spiro-mamakone A
(racemic)



3.2
spiro-mamakone B



3.3
spiro-mamakone C



3.4 and **3.5**
spiro-mamakone D and E
(diastereoisomers, stereochemistry unknown)

Finally, further analogues of the *spiro*-mamakone natural products could be targeted for synthesis. This would provide further information on the structure-activity relationship of the natural metabolites, and could generate analogues with improved biological characteristics.

A deeper understanding of the mechanisms by which the *spiro*-mamakones exert their biological activity would be crucial before any clinical applications for these metabolites could be considered. It would also allow more rational analysis of the structure-activity relationship of synthetic analogues, by excluding those which act via different mechanisms. With accurate structure-activity relationship data and the biological target of the *spiro*-mamakones pin-pointed, rational drug design could also be undertaken. Only with this information could the true viability of these natural products as pharmaceuticals be explored.

3.5 Conclusion

The synthesis of *spiro*-mamakone A was attempted in this project, a task which was, ultimately unsuccessful. However, a number of different synthetic strategies were examined and the limits of these various approaches to the synthetic target were explored. Significant progress was made towards the natural product and the reactivity of these systems better understood.

Beyond the valuable synthetic information gleaned, a substantial number of analogues of the natural product were successfully synthesised. These were all subjected to biological analysis which allowed the structure-activity relationship profile of the natural products to be examined. This analysis confirmed that these natural products are finely-tuned biological entities, with many close analogues displaying no activity under the assay conditions. The importance of the enedione motif, in particular, was brought to light by these results.

In conclusion, whilst the synthesis of the final target natural product was not completed, significant progress was achieved and useful information regarding the natural product's biological activity was obtained as a result of these studies.

3.5 References for Chapter 3

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Chapter 4

Conclusions

The primary aim of this thesis was to use synthetic chemistry as a tool to examine various aspects of the natural products discovered by the University of Canterbury natural products research group. Two projects were undertaken. The first involved the use of synthesis in order to establish the stereochemistry of the pteratides, a series of potently cytotoxic depsipeptides, discovered by Miss Chen. The second project involved an attempt to carry out the total synthesis of *spiro*-mamakone A, another potently cytotoxic fungal natural product, whose structure was already known. The generation of a library of analogues was also undertaken, and an examination of the biological activities of this library was used to probe the structure-activity relationship.

The stereochemical assignment of the pteratides involved the synthesis of a number of unusual amino acids for use as reference materials. The four diastereoisomers of *N*-methylthreonine and 4-methylproline, as well as *N*-methyl-L-valine were all prepared by either following literature preparations, or by modifications thereof. Modifications made to the literature synthesis of the 4-methylproline diastereoisomers led to a more concise synthesis, which gave good stereoselectivity for both enantiomeric pairs, and therefore provided an improved method, compared to literature procedures. These reference materials, as well as commercial amino acids and natural product hydrolysates, were derivatised and analysed by HPLC chromatography to establish the full stereochemistry of each of the depsipeptides in the pteratide series. This work allowed the publication of the pteratides in the peer-reviewed literature.

The total synthesis of *spiro*-mamakone A could not be achieved in the time-frame of this thesis. However, very advanced intermediates were synthesised, and only the generation of a key enedione motif eluded the synthesis of the target natural product. A large

number of analogues which shared the *spiro*-mamakone carbon skeleton, but varied in their degree of oxidation, were generated. The biological activity of this library of analogues was examined, with several analogues showing appreciable cytotoxicity. As well as establishing a viable route to the *spiro*-mamakone skeleton, this project has therefore also given valuable insight into the structure-activity relationship of the *spiro*-mamakone series.

The aims of this thesis were, therefore, largely successfully completed.

Chapter 5

Experimental

5.1 General Methods

Nuclear Magnetic Resonance

The ^{1}H, COSY, NOESY, HSQC and HMBC NMR spectra were recorded on a Varian INOVA 500 NMR spectrometer, at 23 ºC, operating at 500 MHz. The INOVA was equipped with a variable temperature and indirect broadband PFG (pulsed field gradient) 5 mm probe. The ^{13}C NMR spectra were recorded on a Varian UNITY 300 NMR spectrometer, at 23 ºC, operating at 75 MHz. The UNITY was equipped with a variable temperature direct broadband 5 mm probe. Chemical shifts are expressed in parts per million (ppm) on the δ scale, and were referenced to the appropriate solvent peaks: $CDCl_3$ referenced to C**H**Cl_3 at δ_H 7.26 (^{1}H) and **C**DCl_3 at δ_C 77.0 (^{13}C), CD_3OD referenced to C**H**$_3OD$ at δ_H 3.3 (^{1}H) and **C**H_3OD at δ_C 49.3 (^{13}C) and D_2O referenced to **H**OD at δ_H 4.7 (^{1}H) and (**C**$H_3)_3COH$ (added ^{t}BuOH) δ_C 30.3 (^{13}C).

Mass Spectrometry

Samples were analysed on a micromass LCT mass spectrometer equipped with an electrospray ionisation (ESI) probe. A probe voltage of 3200 V at 150 ºC with a nebuliser gas flow of 160 L/hr and desolvation gas flow of 520 L/hr with the source temperature at 80 ºC. The cone voltage was typically 20 V. The solvent flow from a syringe pump in direct injection ESIMS mode was 20 μL/min.

High Pressure Liquid Chromatography

Analytical High Pressure Liquid Chromatography (HPLC) was carried out on two HPLC instruments. The bulk of the work was carried out on a Dionex HPLC instrument using reverse phase chromatography on a Prodigy C_{18} column (250 x 4.6 mm, 5 μm). The column was maintained at a constant temperature (stated in each case) by a Dionex TCC-100 thermostatted column compartment. The samples were eluted at a flow rate of 1 mL/min. A photodiode array (PDA, Dionex UVD340) and Evaporative Light Scattering Detector (ELSD, Dionex ELSD800) were used for detection. Some of the work on the stereochemical assignment of pteratide I (**Section 2.4**, stated in the text) was carried out on a Shimadzu LC-10AD VP liquid chromatograph, using a Phenomenex Prodigy C_{18} column (5μ, 250 x 4.6 mm). The samples were eluted at a flow rate of 1 mL/min and an SPD-M10A VP photodiode array detector was used for detection.

The solvents used were either mixtures of MeCN (Scharlau/LEDA MeCN, HPLC/spectroscopy grade) or MeOH (Merck LiChrosolv) with H_2O (purified using a MilliQ deionising system). The aqueous phase was acidified with 0.05% TFA (Scharlau, synthesis grade) where possible. Elution conditions (eg column temperature, solvents proportions) and retention times are given throughout this chapter.

Column chromatography

All column chromatography was performed as 'flash' chromatography under oxygen-free N_2 gas pressure (~ 1.5 bar) in glass columns. Solvents of commercial grade were used, distilled once in glass distillation apparatus except MeOH which was distilled twice.

Silica flash chromatography was performed on Merck silica gel 60 (230-400 mesh). Reverse phase C_{18} chromatography was performed on Bakerbond® C_{18} (40 μm preparative LC packing). Normal phase diol chromatography was performed on Bakerbond® Diol 40 μm Prep LC packing.

Thin Layer Chromatography

All analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} plastic backed sheets (250 μm thickness). The solvent(s) used for development are indicated in each case. Visualisation was performed first visually under normal light followed by short wavelength UV (254 nm). Staining was carried out using either phosphomolybdic acid spray (PMA, 10% w/v phosphomolybdic acid in ethanol) or ninhydrin dip (1 g in 500 mL MeOH).

IR spectroscopy

IR measurements were taken on either a Shimadzu FTIR-8201PC Spectrophotometer or a Perkin Elmer Spectrum One FTIR Spectrophotometer. Solid samples were measured by diffuse reflectance in KBr, and liquid samples were measured on KBr disks, with a background measured first in all instances.

Optical Rotation

Rotation values were obtained on a Perkin Elmer 341 polarimeter at 20 ºC at a wavelength of 589 nm. The optical rotations were then calculated using the following equation:

$$[\alpha]^{20}_{D} = \alpha / LC$$

where L is the cell path length (dm) and C is the concentration (g/mL)

Biological Assays

Compounds were assayed for cytotoxicity, where applicable, using the P388 MTT antitumour assay. It consists of a 2-fold dilution series of the sample of interest followed by incubation for 72 hours with P388 (murine leukaemia) cells. The concentration of the sample required to reduce the P388 cell growth by 50% (compared to control cells) is determined using the absorbance values obtained when the yellow MTT tetrazolium is reduced by healthy cells to produce the purple colour MTT formazan. The result is expressed as the IC_{50} (ng/mL)

Pteratide hydrolyses and Marfey's derivatisation

The peptides (0.5 mg each) were hydrolyzed by heating (110°C for 24 h) in HCl (6 M; 1 mL). After cooling, the solutions were evaporated to dryness and redissolved in H_2O (100 μL). A 1% (w/v) solution (100 μL) of FDAA (Marfey's reagent, N^{α}-(2,4-dinitro-5-fluorophenyl)-L-alaninamide) in acetone was added to an aliquot (50 μL) of a 50 mM solution of each amino acid or natural product hydrolysate. After addition of $NaHCO_3$ solution (1 M; 20 μL), the mixture was incubated (1 hour at 40 ºC). The reaction was stopped by addition of HCl (2 M, 10 μL), the solvents were evaporated to dryness, and the residue was redissolved in MeOH-H_2O (1:1; 1 mL). An aliquot of each of these solutions (10-50 μL) was analyzed by HPLC.

Solvents

All technical grade solvents were distilled prior to use and MeOH was distilled twice. Dry solvents were obtained using the following standard methods. Toluene, benzene and pyridine were refluxed over calcium hydride and distilled immediately prior to use. THF was refluxed over sodium metal and benzophenone before distillation directly prior to use. DMF was treated twice overnight with activated 4Å molecular sieves, followed by storage over 4Å molecular sieves. MeOH was refluxed over magnesium metal activated with iodine before distillation immediately prior to use. Chloroform was refluxed over P_2O_5 and distilled immediately prior to use.

5.2 Work described in Chapter 2

Section 2.2.2

Fmoc-L-valine, 2.4[1]

L-Valine (200 mg, 1.71 mmol) was dissolved in dioxane (2 mL) and H_2O (2 mL), $NaHCO_3$ (290 mg, 3.42 mmol, 2 eq) was added and the mixture was cooled to 0 ºC. FmocCl (490 mg, 1.89 mmol, 1.1 eq) was then added and the reaction stirred at 0 ºC for 1 hour before being warmed to room temperature and stirred overnight. The reaction was acidified by addition of 1 M $HCl_{(aq)}$ and extracted with ethyl acetate (3 x 5 mL). The combined organic phases were dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. The crude product was then purified by chromatography on silica using a stepwise gradient of 50 to 100% ethyl acetate in petroleum ether to give the product (**2.4**, 382 mg, 1.13 mmol, 66%) as a white solid.

R_f 0.41 (silica, 1:1 petroleum ether:ethyl acetate, visualization UV/PMA); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.77 (2H, d, 7.2 Hz), 7.60 (2H, m), 7.40 (2H, t, 7.0 Hz), 7.32 (2H, t, 7.0 Hz), 5.26 (1H, br d, 8.7 Hz), 4.43 (2H, d, 6.9 Hz), 4.36 (1H, dd, 9.2 Hz, 4.7 Hz), 4.24 (1H, t, 6.9 Hz), 2.25 (1H, m), 1.02 (3H, d, 6.7 Hz), 0.96 (3H, d, 6.7 Hz).

Fmoc-L-valine-oxazolidinone, 2.5[1]

Fmoc-L-valine (**2.4**, 1.03 g, 3.04 mmol) was dissolved in toluene (62 mL) and pTSA (62 mg, 0.33 mmol, 0.1 eq) and paraformaldehyde (618 mg, ~21 mmol, ~35 eq) were added. The mixture was refluxed with a Dean-Stark condenser for 1 hour. After cooling to room temperature, the solvent was removed *in vacuo* and the crude product purified by chromatography on silica using a stepwise gradient of 5% to 20% ethyl acetate in petroleum ether. This gave the product (**2.5**, 1.0 g, 2.85 mmol, 94%) as a colourless oil.

R_f 0.40 (silica, 4:1 petroleum ether:ethyl acetate, visualization UV/PMA); IR (thin film) ν_{max} 1801, 1716, 1418, 1240 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.77 (2H, d, 7.5 Hz), 7.54 (2H, d, 7.5 Hz), 7.42 (2H, dt, 7.5 Hz, 3.3 Hz), 7.33 (2H, dd, 7.5 Hz, 2.4 Hz, 1.0 Hz), 5.45 (1/2H, br), 5.25 (1/2H, br), 5.02 (1H, d, 4.7 Hz), 4.70 (2H, br), 4.23 (1H, br), 4.16 (1/2 H, br), 3.60 (1/2H, br), 2.36 (1/2H, br), 1.72 (1/2H, br), 1.1-0.6 (6H, br); ^{13}C NMR

($CDCl_3$, 75 MHz) δ 171.3, 143.3, 141.4, 128.0, 127.2, 124.5, 124.2, 120.1, 59.9, 47.2, 30.9, 17.7, Fmoc *C*=O not observed.

Fmoc-*N*-methyl-L-valine, 2.6[1]

Fmoc-L-valine-oxazolidinone (**2.5**, 110 mg, 0.31 mmol) was dissolved in chloroform (1.6 mL) and TFA (1.6 mL), and triethylsilane (160 μL, 1.00 mmol, 3.2 eq) was added. The reaction was stirred at room temperature for 30 hours. The solvents were removed *in vacuo* and the crude product purified by chromatography on silica using a stepwise gradient from 20% ethyl acetate in petroleum ether to ethyl acetate. This gave the product (**2.6**, 84 mg, 0.24 mmol, 77%) as a white solid.

R_f 0.18 (silica, 2:1 petroleum ether:ethyl acetate, visualization UV/PMA); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.77 (2H, d, 7.5 Hz), 7.59 (2H, m), 7.41 (2H, t, 7.5 Hz), 7.32 (2H, t, 7.5 Hz), 4.50 (2H, m), 4.28 – 4.10 (2H, m), 2.91 (2H, s), 2.89 (1H, s), 2.30 (2/3H, br), 2.08 (1/3H, br), 1.04 (2H, d, 6.8 Hz), 0.94 (1H, d, 6.8 Hz), 0.90 (2H, d, 6.8 Hz), 0.76 (1H, d, 6.8 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 175.5, 175.2, 157.7, 156.5, 143.7, 143.6, 141.4, 127.8, 127.1, 124.9, 124.8, 120.0, 68.1, 67.9. 65.3, 47.2, 47.1, 31.6, 30.5, 27.4, 19.6, 19.0, 18.8; HRESIMS *m/z* = 354.1698 $[M+H]^+$ 2.0 ppm (354.1705 calcd for $C_{21}H_{24}NO_4$).

***N*-Methyl-L-valine, 2.7**[1]

Fmoc-*N*-methyl-L-valine (**2.6**, 52 mg, 0.15 mmol) was dissolved in MeOH (4 mL) and piperidine (1 mL) and stirred at room temperature for 2 hours. The solvents were removed *in vacuo*, then the product was suspended in H_2O (4 mL) and extracted with chloroform (2 x 3 mL). The aqueous phase was dried down *in vacuo* to give the product (**2.6**, 19 mg, 0.15 mmol, 100%) as a white solid.

$[\alpha]^{20}_D$ = +27 (c 0.5, 6M $HCl_{(aq)}$; lit c 1.0, 6M $HCl_{(aq)}$ + 33.7º);[2] ^{1}H NMR (D_20, 500 MHz) δ 3.30 (1H, d, 4.8 Hz), 2.60 (3H, s), 2.11 (1H, m), 0.94 (3H, d ,7.2 Hz), 0.91 (3H, d, 7.2 Hz);[3] HRESIMS *m/z* = 132.1018 $[M+H]^+$ 5 ppm (132.1025 calcd for $C_6H_{14}NO_2$).

Section 2.2.3

_N_-Benzyl-L-threonine methyl ester, 2.8[4]

L-Threonine methyl ester hydrochloride (1 g, 5.90 mmol) was dissolved in MeOH (10 mL), TEA (825 µL, 5.90 mmol, 1 eq) and benzaldehyde (730 µL, 7.10 mmol, 1.2 eq) were added and the reaction was stirred at room temperature for 1 hour. It was then cooled to 0 ºC and $NaBH_4$ (225 mg, 5.90 mmol, 1 eq) was added. The reaction was stirred for a further 15 minutes at 0 ºC, acidified slowly with 6M $HCl_{(aq)}$, then solvents were removed *in vacuo*. H_2O (10 mL) was added, the mixture basified using 5M $NaOH_{(aq)}$ and extracted with ethyl acetate (3 x 10 mL). The combined organic phases were dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. The crude material was then purified by chromatography on silica using a stepwise gradient of DCM to 5% MeOH in DCM to give the product (**2.8**, 1.22 g, 5.46 mmol, 93%) as a yellow oil. R_f 0.67 (silica, 19:1 DCM:MeOH, visualization UV/PMA); $[\alpha]^{20}_D$ = -49 (c 1.0, $CHCl_3$; lit. c 1.0, MeOH, -34.5º);[4] ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.35-7.25 (5H, m, Bn), 3.84 (1H, d, 13.0 Hz, Bn), 3.71 (3H, s, OC**H**$_3$), 3.69-3.66 (2H, m, H3 and Bn), 3.04 (1H, d, 7.5 Hz, H2), 1.20 (3H, d, 6.2 Hz, H4); HRESIMS *m/z* = 224.1292 $[M+H]^+$ 2.2 ppm (224.1287 calcd for $C_{12}H_{18}NO_3$).

_N_-Benzyl-D-threonine methyl ester, *ent*-2.8[4]

Slightly yellow oil obtained (*ent*-**2.8**, 78 % yield).
$[\alpha]^{20}_D$ = +49 (c 1.0, $CHCl_3$); HRESIMS *m/z* = 224.1296 $[M+H]^+$ 4.0 ppm (224.1287 calcd for $C_{12}H_{18}NO_3$).

_N_-Benzyl-_N_-methyl-L-threonine methyl ester, 2.9[4]

N-Benzyl-L-threonine methyl ester (**2.8**, 1.0 g, 4.43 mmol) was dissolved in MeCN (17 mL), formaldehyde (37%, 1.58 mL, 18.8 mmol, 4.24 eq), $NaBH_3CN$ (304 mg, 4.43 mmol, 1.0 eq) and acetic acid (347 mL, 5.99 mmol, 1.35 eq) sequentially and the reaction was stirred at room temperature for 1 hour. H_2O (20 mL) was added and the mixture basified using 5 M $NaOH_{(aq)}$, then extracted with ethyl acetate (3 x 20 mL). The combined organic phases were dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. The

crude product was then purified by chromatography on silica using 20% ethyl acetate in petroleum ether to give the product (**2.9**, 838 mg, 3.53 mmol, 80%) as a colourless oil. R_f 0.61 (silica, 4:1 petroleum ether:ethyl acetate, visualization UV/PMA); $[\alpha]^{20}_D$ = -98 (c 1.0, MeOH; lit. c 1.0, MeOH, -111.7º);[4] ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.35-7.26 (5H, m, Bn), 3.98 (1H, m, H3), 3.82 (1H, d, 13.2 Hz, Bn), 3.78 (3H, s, OC**H**$_3$), 3.72 (~1H, br s, O**H**), 3.58 (1H, d, 13.2 Hz, Bn), 3.04 (1H, d, 9.9 Hz, H2), 2.28 (3H, s, NC**H**$_3$), 1.17 (3H, d, 6.0 Hz, H4); ^{13}C NMR ($CDCl_3$, from HSQC on 500 MHz) δ 129.0 (CH, Bn), 128.5 (CH, Bn), 127.5 (CH, Bn), 71.9 (CH, C2), 63.0 (CH, C3), 59.5 (CH_2, Bn), 51.2 (CH_3, O**C**H$_3$), 37.8 (CH_3, N**C**H$_3$), 19.3 (CH_3, C4); HRESIMS *m/z* = 238.1432 $[M+H]^+$ 4.6 ppm (238.1443 calcd for $C_{13}H_{20}NO_3$).

_N_-Benzyl-_N_-methyl-D-threonine methyl ester, _ent_-2.9[4]

Colourless oil obtained (*ent*-**2.9**, 77 %).

$[\alpha]^{20}_D$ = +96 (c 1.0, MeOH); HRESIMS *m/z* = 238.1443 $[M+H]^+$ 0 ppm (238.1443 calcd for $C_{13}H_{20}NO_3$).

_N_-Methyl-L-threonine methyl ester, 2.10[4]

N-Benzyl-*N*-methyl-L-threonine methyl ester (**2.9**, 55 mg, 0.20 mmol) was dissolved in MeOH (2 mL) and Pd/C (5 mg) was added. The reaction was placed under a $H_{2(g)}$ atmosphere (using a balloon) and the reaction was stirred at room temperature for 20 hours. The catalyst was removed by filtration of the mixture though a short celite pad using DCM (50 mL) and the solvents were removed *in vacuo* to give the product (**2.10**, 16 mg, 0.11 mmol, 55%) as a colourless oil.

$[\alpha]^{20}_D$ = -19 (c 1.0, MeOH; lit. c 1.0, MeOH, -18.7º);[4] ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.77 (3H, s, OC**H**$_3$), 3.66 (1H, m, H3), 2.91 (3H, d, 7.7 Hz, H2), 2.42 (3H, s, NC**H**$_3$), 1.21 (3H, d, 6.2 Hz, H4); HRESIMS *m/z* = 148.0973 $[M+H]^+$ 0.7 ppm (148.0974 calcd for $C_6H_{14}NO_3$).

_N_-Methyl-D-threonine methyl ester, _ent_-2.10[4]

Colourless oil obtained (_ent_-**2.10**, 97 % yield).

$[\alpha]^{20}_{D}$ = +17 (0.9, MeOH); HRESIMS _m/z_ = 148.0980 $[M+H]^+$ 4.1 ppm (148.0974 calcd for $C_6H_{14}NO_3$).

_N_-Methyl-L-threonine, 2.11[4]

_N_-Methyl-L-threonine methyl ester (**2.10**, 130 mg, 0.88 mmol) was dissolved in THF (10 mL) and 0.1 M $LiOH_{(aq)}$ (8.8 mL, 0.88 mmol, 1 eq) and stirred at room temperature overnight. The solvents were removed _in vacuo_ to give the product as a white amorphous solid (**2.11**, ~ 100%).

$[\alpha]^{20}_{D}$ = -2 (c 1.0, H_2O); ^{1}H NMR (D_2O, 500 MHz) δ 3.68 (1H, m, H3) 2.72 (1H, d, 6.7 Hz, H2), 2.15 (3H, s, NC**H**$_3$), 1.05 (3H, 6.5 Hz, H4);[5] HRESIMS _m/z_ = 134.0821 $[M+H]^+$ 3.0 ppm (134.0817 calcd for $C_5H_{12}NO_3$).

_N_-Methyl-D-threonine, _ent_-2.11[4]

White amorphous solid (~100%).

$[\alpha]^{20}_{D}$ = +10 (c 1.0, H_2O)

Section 2.2.4

CBz-_N_-methyl-L-threonine, 2.12

_N_-Methyl-L-threonine methyl ester (**2.10**, 62 mg, 0.34 mmol) was dissolved in dioxane (1 mL), cooled in an ice-bath and $NaHCO_{3(aq)}$ (1M, 335 µL, 0.34 mmol, 1 eq) was added. CBzCl (76 µL, 0.54 mmol, 1.6 eq) was added slowly, with simultaneous addition of $NaHCO_{3(aq)}$ (1M, 335 µL, 0.34 mmol, 1 eq). The reaction was allowed to warm to room temperature and stirred for 16 hours. After removal of most of the solvent _in vacuo_, the mixture was partitioned between ethyl acetate and H_2O. The organic phase was then dried over $MgSO_4$, filtered and the solvent removed _in vacuo_. The crude material was purified by chromatography on silica using a step-wise gradient from 5 to 50% ethyl acetate in petroleum ether, to give the correct product (**2.12**, 66 mg, 0.25 mmol, 74%) as a colourless oil.

R_f 0.36 (silica, 1:1 petroleum ether:ethyl acetate, visualization UV/PMA); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.38-7.31 (5H, m, CBz), 5.18 (2H, br s, CBz), 4.61 (1H, d, 4.8 Hz, H2), 4.45 (1H, m, H3), 3.77 (3H, s, OC**H**$_3$), 3.03 (3H, s, N C**H**$_3$), 1.25 (3H, d, 6.5 Hz, H4); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.6 (C, C=O), 157.6 (C, CBz), 136.4 (C, CBz), 128.5 (CH, CBz), 128.0 (CH, CBz), 127.7 (CH, CBz), 67.7 (CH_2, CBz), 67.2 (CH, C3), 64.5 (CH, C2), 52.4 (CH_3, O**C**H_3), 34.0 (CH_3, N **C**H_3), 19.5 (CH_3, C4); HRESIMS *m/z* = 282.1339 $[M+H]^+$ 0.7 ppm (282.1341 calcd for $C_{14}H_{20}NO_5$).

Dehydration of CBz-*N*-methyl-L-threonine methyl ester, 2.11, to give 2.13

CBz-*N*-methyl-L-threonine methyl ester (**2.11**, 30 mg, 0.11 mmol) was dissolved in dry pyridine (1 mL), cooled in an ice-bath and tosic anhydride (81 mg, 0.22 mmol, 2 eq) was added. The reaction was allowed to warm slowly to room temperature and stirred for 16 hours. After removal of the solvent *in vacuo*, the crude residue was purified by chromatography on silica using a step-wise gradient of 10 to 40% ethyl acetate in petroleum ether. Starting material (9.3 mg, 0.033 mmol, 30%) and the dehydration product (**2.13**, 9.3 mg, 0.035 mmol, 32%, colourless oil) were obtained.
R_f 0.75 (silica, 1:1 petroleum ether:ethyl acetate, visualization UV/PMA); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.41-7.25 (5H, m, CBz), 6.92 (1/4H, q, 7.1 Hz, H3), 6.83 (3/4H, q, 7.1 Hz, H3), 5.19 (1/2H, br s, CBz), 5.10 (3/2H, br s, CBz), 3.76 (3/4H, s, OC**H**$_3$), 3.64 (9/4H, s, OC**H**$_3$), 3.05 (3/4H, s, NC**H**$_3$), 3.04 (9/4H, s, NC**H**$_3$), 1.80 (3/4H, d, 7.1 Hz, H4), 1.76 (9/4H, d, 7.1 Hz, H4); HRESIMS *m/z* = 264.1231 $[M+H]^+$ 1.9 ppm (264.1236 calcd for $C_{14}H_{18}NO_4$).

***N*-Benzyl-*N*-methyl-*O*-acetyl-L-threonine methyl ester, 2.15**

N-Benzyl-*N*-methyl-L-threonine methyl ester (**2.9**, 25 mg, 0.11 mmol) was dissolved in anhydrous THF, DEAD (71 μL, 0.45 mmol, 4.1 eq), dry acetic acid (100 μL, 1.75 mmol, 16 eq) and PPh_3 (118 mg, 0.45 mmol, 4.1 eq) were added and the reaction was stirred at room temperature for 16 hours. After removal of solvents *in vacuo*, the crude residue was purified by chromatography on silica using a step-wise gradient of 1 to 20% ethyl acetate in petroleum ether. Starting material (**2.9**, 12.6 mg, 0.053 mmol, 48%) and product (**2.15**, 15.6 mg, 0.056 mmol, 51%, colourless oil) were obtained.

R_f 0.43 (silica, 4:1 petroleum ether:ethyl acetate, visualization UV/PMA); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.31-7.22 (5H, m, Bn), 5.40 (1H, m, H3), 3.83 (1H, d, 13.6 Hz, Bn), 3.75 (3H, s, OC**H**$_3$), 3.64 (1H, d, 13.6 Hz, Bn), 3.36 (1H, d, 8.5 Hz, H2), 2.31 (3H, s, NC**H**$_3$), 2.08 (3H, s, COC**H**$_3$), 1.24 (3H, d, 6.3 Hz, H4); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.2 (C=O x 2), 128.6(Bn), 128.2 (Bn), 127.1 (Bn), 69.4 (C2 or C3), 67.7 (C2 or C3), 59.5 (Bn), 51.2 (O**C**H$_3$), 38.5 (N**C**H$_3$), 21.3 (CO**C**H$_3$), 17.6 (C4), quartenary benzyl C not observed; HRESIMS *m/z* = 280.1557 $[M+H]^+$ 2.9 ppm (280.1549 calcd for $C_{15}H_{22}NO_4$).

_N_-Methyl-_O_-acetyl-L-threonine methyl ester, 2.16

N-Benzyl-*N*-methyl-*O*-acetyl-L-threonine methyl ester (**2.15**, 44 mg, 0.16 mmol) was dissolved in MeOH (2 mL) and palladium on carbon (10% w/w, 4 mg) was added. The reaction was then placed under an atmosphere of $H_{2(g)}$ and stirred at room temperature for 20 hours. The reaction was filtered through a pad of celite using DCM (20 mL) and the solvents were removed *in vacuo*. ^{1}H NMR spectrum of the crude product suggested the correct product had been formed, but impurities were present. The crude material was taken up in $HCl_{(aq)}$ (1 M, 2 mL) and extracted with ethyl acetate (2 x 2 mL). The aqueous phase was basified with $NaHCO_{3(aq)}$ (1 M) and extracted with ethyl acetate (3 x 2 mL), the combined organic phases were dried over $MgSO_4$, filtered and the solvents removed *in vacuo*. ^{1}H NMR spectrum showed the product was still very impure.
^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.03 (1 H, d, 4.4 Hz, H2), 4.48 (1H, m, H3), 3.77 (3H, s, OC**H**$_3$), 3.12 (3H, s, NC**H**$_3$), 2.18 (3H, s, COC**H**$_3$), 1.21 (3H, d, 6.7 Hz, H4)

_N_-Benzyl-_N_-methyl-_O_-acetyl-L-threonine, 2.17

N-Benzyl-*N*-methyl-*O*-acetyl-L-threonine methyl ester (**2.15**, 15 mg, 0.054 mmol) was dissolved in THF (1.6 mL) and $LiOH_{(aq)}$ (0.1M, 1.6 mL, 0.16 mmol, 3 eq), and stirred at room temperature for 16 hours. The solvents were then removed *in vacuo* to give the product (**2.17**, 17.2 mg obtained – contains salt impurities).
^{1}H NMR (D_2O, 500 MHz) δ 7.31-7.22 (5H, m, Bn), 3.87 (1H, m, H3), 3.73 (1H, d, 13.0 Hz, Bn), 3.43 (1H, d, 13.0 Hz, Bn), 2.82 (1H, d, 9.9 Hz, H2), 2.14 (3H, s, NC**H**$_3$), 1.80 (3H, s, COC**H**$_3$), 1.05 (3H, d, 6.2 Hz, H4); ^{13}C NMR (CD_3OD, 75 MHz) δ 182.7 (C, C=O), 178.2 (C, C=O), 140.2 (C, Bn), 130.9 (CH, Bn), 129.7 (CH, Bn), 128.6 (CH, Bn),

76.6 (CH, C2), 65.7 (CH, C3), 59.6 (CH_2, Bn), 39.1 (CH_3, N**C**H_3), 24.5 (CH_3, CO**C**H_3), 20.3 (CH_3, C4).

***N*-Benzyl-*N*-methyl-*O*-benzoyl-L-threonine methyl ester, 2.18**

Colourless oil obtained (**2.18**, 84 %). Full analytical data given for *ent*-**2.18** (see below). $[\alpha]^{20}_D$ = -78 (c 1.0, $CHCl_3$).

***N*-Benzyl-*N*-methyl-*O*-benzoyl-D-threonine methyl ester, *ent*-2.18**

N-Benzyl-*N*-methyl-D-threonine methyl ester (**2.9**, 50 mg, 0.22 mmol) was dissolved in anhydrous THF, DEAD (142 μL, 0.90 mmol, 4.1 eq), benzoic acid (516 mg, 4.22 mmol, 19 eq) and PPh_3 (236 mg, 0.90 mmol, 4.1 eq) were added and the reaction was stirred at room temperature for 60 hours. After removal of solvents *in vacuo*, the crude residue was purified by chromatography on silica using a step-wise gradient of 2 to 20% ethyl acetate in petroleum ether to give the product (*ent*-**2.18**, 71 mg, 0.21, 9%) as a colourless oil. R_f 0.38 (silica, 4:1 petroleum ether:ethyl acetate, visualization UV/PMA); $[\alpha]^{20}_D$ = +75 (c 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.03 (2H, d, 7.8 Hz, Bz), 7.58 (1H, t, 7.7 Hz, Bz), 7.46 (2H, t, 7.8 Hz, Bz), 7.26-7.19 (5H, m, Bn), 5.66 (1H, m, H3), 3.85 (1H, d, 13.6 Hz, Bn), 3.75 (3H, s, OC**H**$_3$), 3.70 (1H, d, 13.6 Hz, Bn), 3.49 (1H, d, 8.3 Hz, H2), 2.40 (3H, s, NC**H**$_3$), 1.37 (3H, d, 6.3 Hz, H4); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.2 (C, C=O), 165.7 (C, C=O), 139.1 (C, Bn), 132.9 (CH, Bz), 129.7 (CH, Bz), 128.7 (CH, Bn), 128.3 (CH, Bz), 128.1 (CH, Bn), 127.0 (CH, Bn), 69.2 (CH, C2), 68.5 (CH, C3), 59.7 (CH_2, Bn), 51.2 (CH_3, O**C**H_3), 38.4 (CH_3, N**C**H_3), 17.6 (CH_3, C4); HRESIMS *m/z* = 342.1718 $[M+H]^+$ 3.8 ppm (342.1705 calcd for $C_{20}H_{24}NO_4$).

***N*-Methyl-*O*-benzoyl-L-threonine methyl ester, 2.19**

Colourless oil obtained (**2.19**, 84 %). Full analytical data given for *ent*-**2.19** (see below). $[\alpha]^{20}_D$ = -20 (c 1.0, $CHCl_3$).

_N_-Methyl-_O_-benzoyl-D-threonine methyl ester, *ent*-**2.19**

N-Benzyl-*N*-methyl-*O*-benzoyl-D-threonine methyl ester (*ent*-**2.18**, 10 mg, 0.029 mmol) was dissolved in ethyl acetate (1 mL), Pd/C (2 mg) was added and the reaction placed under an H_2 atmosphere by use of a balloon. The reaction was stirred at room temperature for 16 hours, then filtered through a short celite pad with DCM (~ 20 mL). After removal of the solvents *in vacuo* the product (*ent*-**2.19**, 5.5 mg, 0.022 mmol, 76%) was obtained as a colourless oil.

R_f 0.16 (silica, 4:1 petroleum ether:ethyl acetate, visualization UV/ninhydrin); $[\alpha]^{20}_D$ = +16 (c 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.01 (2H, d, 7.9 Hz, Bz), 7.55 (1H, t, 7.5 Hz, Bz), 7.43 (2H, t, 7.7 Hz, Bz), 5.57 (1H, m, H3), 3.71 (3H, s, OC**H**$_3$), 3.38 (1H, d, 7.9 Hz, H2), 2.41 (3H, s, NC**H**$_3$), 1.38 (3H, d, 6.4 Hz, H4); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.1 (C, C=O), 165.7 (C, C=O), 133.0 (CH, Bz), 130.1 (C, Bz), 129.6 (CH, Bz), 128.4 (CH, Bz), 71.4 (CH, C3), 66.5 (CH, C2), 52.0 (CH_3, O**C**H$_3$), 35.5 (CH_3, N**C**H$_3$), 16.8 (CH3, C4); HRESIMS *m/z* = 252.1237 $[M+H]^+$ 0.4 ppm (252.1236 calcd for $C_{13}H_{18}NO_4$).

***N*-Methyl-L-threonine**, **2.20**

White amorphous solid obtained (contains salt impurites). Further analytical data given for *ent*-**2.20** (see below).

_N_-Methyl-D-threonine, *ent*-**2.20**

N-Methyl-*O*-benzoyl-D-threonine methyl ester (*ent*-**2.19**, 7 mg, 0.028 mmol) was dissolved in ethanol (200 μL) and 0.5M NaOH(aq) (220 μL, 0.11 mmol, 4 eq). The reaction was stirred at room temperature for 16 hours. The solvents were partially removed *in vacuo*, then the mixture was washed with ethyl acetate (2 x 1 mL), acidified with 1M $HCl_{(aq)}$ and washed again with ethyl acetate (2 x 1 mL) then the solvents were removed *in vacuo* to give the product as a white amorphous solid (*ent*-**2.20**, contains salt impurities).

^{1}H NMR (D_2O, 500 MHz) δ 4.11 (1H, m, H3), 3.58 (1H, d, 6.6 Hz, H2), 2.73 (3H, s, NC**H**$_3$), 1.30 (3H, d, 6.5 Hz, H4); ^{13}C NMR ($CDCl_3$, from HSQC at 500 MHz) δ 68.6 (CH, C2), 66.0 (CH, C3), 32.6 (CH_3, N**C**H$_3$), 19.4 (CH_3, C4); HRESIMS *m/z* = 134.0816 $[M+H]^+$ 0.7 ppm (134.0817 calcd for $C_5H_{12}NO_3$).

Section 2.2.6

L-*allo*-Threonine methyl ester, 2.26

L-*allo*-Threonine (50 mg, 0.42 eq) was suspended in MeOH (2 mL) and thionyl chloride (200 μL, 2.74 mmol, 6.5 eq) was added dropwise. The reaction was then refluxed for 5 hours. After cooling, the solvent and residual thionyl chloride were removed *in vacuo* to give the product (**2.26**, 70 mg, 0.42 mmol, ~100%) as a colourless oil.
^{1}H NMR (D_2O, 75 MHz) δ 4.07 (1H, m, H3), 3.89 (1H, d, 3.4 Hz, O**H**), 3.68 (3H, s, O C**H**$_3$), 3.13 (1H, m, H2), 1.08 (3H, d, 6.6 Hz, H4).

D-*allo*-Threonine methyl ester, *ent*-2.26

Prepared as **2.26**, using D-*allo*-threonine as starting material. Product obtained as colourless oil (~ 100%).

***N*-Benzyl-L-*allo*-threonine methyl ester, 2.27**

Prepared as **2.8**, using **2.26** as starting material. White crystalline solid obtained (**2.27**, 60% yield).
R_f 0.31 (silica, 19:1 DCM: MeOH, visualization UV/PMA); mp 91-93 ºC; $[\alpha]^{20}_D$ = -56 (c 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.35-7.27 (5H, m, Bn), 4.05 (1H, m, H3), 3.92 (1H, d, 12.8 Hz, Bn), 3.76 (3H, s, OC**H**$_3$), 3.69 (1H, d, 12.8 Hz, Bn), 3.42 (1H, d, 4.7 Hz, H2), 1.10 (3H, d, 6.5 Hz, H4); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.3 (C=O), 139.0 (Bn), 128.5 (Bn), 128.4 (Bn), 127.4 (Bn), 67.1 (C2 or C3), 65.3 (C2 or C3), 52.7 (Bn or O**C**H$_3$), 52.0 (Bn or O**C**H$_3$), 18.8 (C4); HRESIMS *m/z* = 224.1288 $[M+H]^+$ 0.4 ppm (224.1287 calcd for $C_{12}H_{18}NO_3$).

***N*-Benzyl-D-*allo*-threonine methyl ester, *ent*-2.27**

Prepared as **2.8**, using *ent*-**2.26** as starting material. White crystalline solid obtained (*ent*-**2.27**, 62 % yield).
$[\alpha]^{20}_D$ = +50 (c 0.4, $CHCl_3$).

***N*-Benzyl-*N*-methyl-L-*allo*-threonine methyl ester, 2.28**

Prepared as **2.9**, using **2.27** as starting material. Colourless oil obtained (**2.28**, 88% yield). R_f 0.17 (silica, 4:1 petroleum ether:ethyl acetate, visualization UV/PMA); $[\alpha]^{20}_D$ = -128 (c 1.0, $CHCl_3$); IR (thin film) ν_{max} 3500-3300, 1732, 1454, 1196, 1173 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.32-7.24 (5H, m, Bn), 4.17 (1H, m, H3), 3.81 (3H, s, OC**H**$_3$), 3.75 (1H, d, 13.6 Hz, Bn), 3.62 (1H, d, 13.6 Hz, Bn), 3.09 (1H, d, 8.7 Hz, H2), 2.24 (3H, s, NC**H**$_3$), 1.30 (3H, d, 6.4 Hz, H4); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.7 (C=O), 138.7 (Bn), 128.8 (Bn), 128.3 (Bn), 127.2 (Bn), 71.5 (C2 or C3), 66.1 (C2 or C3), 60.0 (Bn), 51.2 (O**C**H$_3$), 38.5 (N**C**H$_3$), 20.0 (C4); HRESIMS *m/z* = 238.1439 $[M+H]^+$ 1.7 ppm (238.1443 calcd for $C_{13}H_{20}NO_3$).

***N*-Benzyl-*N*-methyl-D-*allo*-threonine methyl ester**, *ent*-**2.28**

Prepared as **2.9**, using *ent*-**2.27** as starting material. Colourless oil obtained (*ent*-**2.28**, 95% yield).

$[\alpha]^{20}_D$ = +129 (c 1.0, $CHCl_3$).

***N*-Methyl-L-*allo*-threonine methyl ester, 2.29**

Prepared as **2.10**, using *ent*-**2.28** as starting material. Colourless oil obtained (**2.29**, 80% yield).

$[\alpha]^{20}_D$ = -4 (c 1.0, MeOH); IR (thin film) ν_{max} 3400-3200, 1736, 1435, 1173 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 4.04 (1H, m, H3), 3.77 (3H, s, OC**H**$_3$), 3.26 (1H, d, 4.7 Hz, H2), 2.44 (3H, s, NC**H**$_3$), 1.10 (3H, d, 6.5 Hz, H4); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.4 (C=O), 68.0 (C2 or C3), 67.1 (C2 or C3), 51.9 (O**C**H$_3$), 35.4 (N**C**H$_3$), 18.8 (C4); HRESIMS *m/z* = 148.0982 $[M+H]^+$ 5.4 ppm (148.0974 calcd for $C_6H_{14}NO_3$).

***N*-Methyl-D-*allo*-threonine methyl ester,** *ent*-**2.29**

Prepared as **2.10**, using *ent*-**2.28** as starting material. Colourless oil obtained (*ent*-**2.29**, 56% yield).

$[\alpha]^{20}_D$ = +4 (c 0.08, MeOH)

_N_-Methyl-L-_allo_-threonine, 2.30

_N_-Methyl-L-_allo_-threonine methyl ester, (**2.29**, 5 mg, 0.04 mmol) was dissolved in 6M $HCl_{(aq)}$ (1 mL) and the reaction heated to 50 ºC for 16 hours. After cooling to room temperature, the solvent was removed *in vacuo*. White solid obtained (**2.30**, 6 mg, 0.04 mmol, ~ 100%).

$[\alpha]^{20}_D$ = +6 (c 0.4, H_2O); ^{1}H NMR (D_2O, 500 MHz) δ 4.30 (1H, m, H3), 3.86 (1H, d, 3.4 Hz, H2), 2.68 (3H, s, NC**H**$_3$), 1.19 (3H, d, 6.8 Hz, H4); ^{13}C NMR (D_2O, 75 MHz) δ 169.4 (C=O), 65.4 (C2 and C3), 33.0 (N**C**H_3), 18.2 (C4); HRESIMS *m/z* = 134.0823 $[M+H]^+$ 4.5 ppm (134.0817 calcd for $C_5H_{12}NO_3$).

_N_-Methyl-D-_allo_-threonine, ***ent*-2.30**

Prepared as **2.30**, using ***ent*-2.29** as starting material. White solid obtained (***ent*-2.30**, ~ 100%).

Section 2.3.2

_N_-Benzyl-L-_trans_-4-hydroxyproline ethyl ester, 2.34

First esterification was carried out by a literature method to give L-*trans*-4-hydroxyproline ethyl ester hydrochloride as follows:[6]

L-*trans*-4-hydroxyproline (1 g, 7.63 mmol) was suspended in ethanol (10 mL) and thionyl chloride (620 μL, 8.55 mmol, 1.1 eq) was added dropwise with stirring. The reaction was then refluxed for 4 hours, during which time the hydroxyproline dissolved. After cooling to room temperature, the mixture was poured into diethyl ether (100 mL), causing the precipitation of the product. After further cooling to 4 ºC for 30 minutes the precipitate was filtered, washed with diethyl ether (2 x 20 mL) and the remaining solvents were removed *in vacuo* to give the product as a white amorphous solid HCl salt (1.42 g, 7.30 mmol, 96%).

^{1}H NMR (CD_3OD, 500 MHz) δ 4.57 (2H, m), 4.31 (2H, q, 7.0 Hz), 3.40 (1H, dd, 12.2 Hz, 3.4 Hz), 3.31 (obscured by CD_3OH), 2.42 (1H, dd, 13.7 Hz, 8.3 Hz), 2.17 (1H, ddd, 13.7 Hz, 10.6 Hz, 4.3 Hz), 1.32 (3H, t, 7.0 Hz).

L-*trans*-4-hydroxyproline ethyl ester hydrochloride (1.28 g, 6.54 mmol) was then suspended in chloroform (25 mL), TEA (2.13 mL, 15.3 mmol, 2.3 eq) and benzyl bromide (830 μL, 7.0 mmol, 1.05 eq) were added and the reaction was refluxed for 6 hours. After cooling the reaction to room temperature, $NaOH_{(aq)}$ (1M,15 mL) was added, the phases separated, the aqueous phase extracted with DCM (2 x 20 mL) and the combined organic phases dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. The crude product was then purified by chromatography on silica using 30% petroleum ether in ethyl acetate to give the product (1.41 g, 5.64 mmol, 86%) as a slightly yellow oil. R_f 0.46 (silica, 3:7 petroleum ether:ethyl acetate, visualization UV/PMA); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.33-7.24 (5H, m, Bn), 4.46 (1H, m, H4), 4.13 (2H, q, 7.1 Hz, Et), 3.93 (1H, d, 12.9 Hz, Bn), 3.67 (1H, d, 12.9 Hz, Bn), 3.60 (1H, t, 7.8 Hz, H2), 3.32 (1H, dd, 10.2 Hz, 5.6 Hz, H5a), 2.48 (1H, dd, 10.2 Hz, 3.8 Hz, H5b), 2.26 (1H, dt, 13.4 Hz, 7.1 Hz, H3a), 2.08 (1H, ddd, 13.4 Hz, 8.2 Hz, 3.4 Hz, H3b), 1.25 (3H, t, 7.2 Hz, Et); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.5 (C, C=O), 138.1 (C, Bn), 129.0 (CH, Bn), 128.3 (CH, Bn), 127.2 (CH, Bn), 70.4 (CH, C4), 63.6 (CH, C2), 61.1 (CH_2, C5), 60.6 (CH_2, Et), 58.0 (CH_2, Bn), 39.6 (CH_2, C3), 14.2 (CH_3, Et); HRESIMS *m/z* = 250.1445 $[M+H]^+$ 0.8 ppm (250.1443 calcd for $C_{14}H_{20}NO_3$).

_N_-Benzyl-_O_-tosyl-L-_trans_-4-hydroxyproline ethyl ester, 2.35[6]

N-Benzyl-L-*trans*-4-hydroxyproline ethyl ester (**2.34**, 150 mg, 0.60 mmol) was dissolved in dry pyridine (3 mL) and cooled in an ice-bath. pTSACl (138 mg, 0.72 mmol, 1.2 eq) was then added, the reaction was allowed to warm to room temperature and stirred for 40 hours. Citric acid (5% w/v, 5 mL) was added and the reaction extracted using diethyl ether (4 x 5 mL). The combined organic phases were dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. The crude product was purified by chromatography on silica using a step-wise gradient of 0 to 20% ethyl acetate in petroleum ether to give the product (**2.35**, 139 mg, 0.34 mmol, 57%) as a yellow oil.

OR

N-Benzyl-L-*trans*-4-hydroxyproline ethyl ester (**2.34**, 500 mg, 2.01 mmol) was dissolved in dry pyridine (10 mL), cooled in an ice-bath and tosic anhydride (1.09 g, 3.01 mmol, 1.5 eq) was added. The reaction was allowed to warm to room temperature and stirred

for 40 hours, then worked up as above to give the product (**2.35**, 382 mg, 0.95 mmol, 48%).

R_f 0.30 (silica, 4:1 petroleum ether:ethyl acetate, visualization UV/PMA); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.75 (2H, d, 8.3 Hz, Ts), 7.33-7.24 (7H, m, Bn and Ts), 4.98 (1H, m, H4), 4.11 (2H, q, 7.1 Hz, Et), 3.88 (1H, d, 12.9 Hz, Bn), 3.60 (1H, d, 12.9 Hz, Bn), 3.55 (1H, t, 7.8 Hz, H2), 3.26 (1H, dd, 11.2 Hz, 6.2 Hz, H5a), 2.64 (1H, dd, 11.2 Hz, 3.9 Hz, H5b), 2.44 (3H, s, Ts), 2.27 (2H, m, H3), 1.23 (3H, t, 7.2 Hz, Et); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.4 (C, C=O), 144.9 (C, Bn or Ts), 137.4 (C, Bn or Ts), 133.5 (C, Bn or Ts), 129.9 (CH, Bn or Ts), 128.9 (CH, Bn or Ts), 128.3 (CH, Bn or Ts), 127.8 (CH, Bn or Ts), 127.3(CH, Bn or Ts), 79.4 (CH, C4), 63.5 (CH, C2), 60.9 (CH_2, Et), 57.9 (CH_2, C5), 57.8 (CH_2, Bn), 36.5 (CH_2, C3), 21.6 (CH_3, Ts), 14.2 (CH_3, Et); HRESIMS *m/z* = 404.1514 $[M+H]^+$ 4.5 ppm (404.1532 calcd for $C_{21}H_{26}NO_5S$).

_N_-Benzyl-_O_-tosyl-L-_cis_-4-hydroxyproline ethyl ester, 2.36

N-Benzyl-L-*trans*-4-hydroxyproline ethyl ester (**2.34**, 150 mg, 0.60 mmol) was dissolved in dry benzene (12 mL), triphenylphosphine (825 mg, 3.2 mmol, 5.3 eq), zinc tosylate (162 mg, 0.40 mmol, 0.7 eq) and DEAD (500 μL, 3.2 mmol, 5.3 eq) were added and the reaction (protected from light) was stirred at room temperature for 3 hours. After removal of the solvent, the crude product was purified by chromatography using a step-wise gradient of 0 to 50% ethyl acetate in petroleum ether to give the product (**2.36**, 126 mg, 0.31 mmol, 52%) as a colourless oil.

R_f 0.69 (silica, 1:1 petroleum ether:ethyl acetate, visualization UV/PMA); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.74 (2H, d, 8.2 Hz, Ts), 7.31-7.23 (7H, m, Bn and Ts), 4.98 (1H, m, H4), 4.30 (1H, m, H2), 4.15 (2H, q, 7.2 Hz, Et), 3.98 (1 H, d, 13.2 Hz, Bn), 3.51 (1H, d, 13.2 Hz, Bn), 3.25 (1H, dd, 8.4 Hz, 6.9 Hz, H2), 3.07 (1H, dd, 11.1 Hz, 2.2 Hz, H5), 2.61 (1H, m, H5), 2.48 (1H, dt, 14.5 Hz, 8.1 Hz, H3a), 2.42 (2H, s, Ts), 2.20 (1H, m, H3b), 1.26 (3H, t, 7.2 Hz, Et); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.2 (C, C=O), 144.7 (C, Ts or Bn), 137.3 (C, Ts or Bn), 133.9 (C, Ts or Bn), 129.8 (CH, Ts or Bn), 129.7 (CH, Ts or Bn), 128.9 (CH, Ts or Bn), 128.3 (CH, Ts or Bn), 127.7 (CH, Ts or Bn), 127.2 (CH, Ts or Bn), 78.7 (CH, C4), 63.3 (CH, C2), 60.9 (CH_2, Et), 57.8 (CH_2, C5), 57.1 (CH_2, Bn), 36.5

(CH_2, C3), 21.6 (CH_3, Ts), 14.2 (CH_3, Et); HRESIMS *m/z* = 404.1533 $[M+H]^+$ 0.2 ppm (404.1532 calcd for $C_{21}H_{26}NO_5S$).

_N_-Benzyl-L-_cis_-4-methylproline ethyl ester, 2.37

Copper (I) iodide (148 mg, 0.77 mmol, 7 eq) was suspended in dry diethyl ether (1 mL) and cooled to 0 ºC in an ice bath. Methyl lithium (1.6 M in diethyl ether, 960 µL, 1.58 mmol, 14 eq) was added dropwise, and the reaction was stirred at 0 ºC for 2 hours. A solution of *N*-benzyl-*O*-tosyl-L-*trans*-4-hydroxyproline ethyl ester (**2.35**, 45 mg, 0.11 mmol) in dry diethyl ether (1 mL) was then added dropwise and stirred at 0 ºC for 8 hours. A saturated solution of $Na_2CO_{3(aq)}$ (3 mL) was added and the aqueous phase was extracted with diethyl ether (3 x 3 mL). The combined organic phases were dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. The crude residue was purified by chromatography on silica using a stepwise gradient of 0 to 50% ethyl acetate in petroleum ether to give the product (**2.37**, 1.4 mg, 0.006 mmol, 5%) as a colourless oil. R_f 0.48 (silica, 4:1 petroleum ether:ethyl acetate, visualization UV/PMA); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.35-7.23 (5H, m, Bn), 4.14 (2H, dq, 7.3 Hz, 2.5 Hz), 3.92 (1H, d, 12.9 Hz, Bn), 3.55 (1H, d, 13.0 Hz, Bn), 3.36 (1H, t, 7.9 Hz, H2), 2.68 (1H, m, H5a), 2.63 (1H, br t, 8.0 Hz, H5b), 2.32 (1H, dt, 12.3 Hz, 8.0 Hz, H3a), 2.24 (1H, m, H4), 1.58 (1H, 12.4 Hz, 7.4 Hz, H3b), 1.25 (3H, t, 7.2 Hz, Et), 1.05 (3H, d, 6.7 Hz, H6); ^{13}C NMR ($CDCl_3$, taken from 2D data at 500 MHz) δ 129.0 (CH, Bn), 128.2 (CH, Bn), 127.1 (CH, Bn), 65.9 (CH, C2), 60.4 (CH_2, Et), 59.9 (CH_2, C5), 58.2 (CH_2, Bn), 31.3 (CH, C4), 20.1 (CH_3, C6), 14.3 (CH_3, Et); LRESIMS *m/z* = 248.2 $[M+H]^+$ (248.2 calcd for $C_{15}H_{22}NO_2$).

_N_-Benzyl-L-_trans_-4-methylproline ethyl ester, 2.38

Copper (I) iodide (114 mg, 0.60 mmol, 7.5 eq) was suspended in dry diethyl ether (500 μL) and cooled to –8 ºC in an MeOH /H_2O/dry ice bath. Methyl lithium (1.6 M in diethyl ether, 700 μL, 1.12 mmol, 14 eq) was added dropwise, and the reaction was stirred at –8 ºC for 120 minutes. A solution of *N*-benzyl-*O*-tosyl-L-*cis*-4-hydroxyproline ethyl ester (**2.36**, 32 mg, 0.08 mmol) in dry diethyl ether (1 mL) was then added dropwise and then stirred at –8 ºC for 8 hours. A saturated solution of $Na_2CO_{3(aq)}$ (3 mL) was added and after warming the mixture to room temperature, it was extracted with diethyl ether (3 x 3 mL). The combined organic phases were dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. The crude residue was purified by chromatography on silica using a stepwise gradient of 0 to 50% ethyl acetate in petroleum ether. The product (**2.38**, 15 mg, 0.06 mmol, 75%) was obtained as a colourless oil.

R_f 0.24 (silica, 4:1 petroleum ether:ethyl acetate, visualization UV/PMA); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.34-7.23 (5H, m, Bn), 4.11 (2H, m, Et), 3.91 (1H, d, 12.7 Hz, Bn), 3.53 (1H, d, 12.7 Hz, Bn), 3.29 (1H, dd, 9.5 Hz, 5.8 Hz, H2), 3.13 (1H, dd, 8.7 Hz, 6.9 Hz, H5a), 2.34 (1H, m, H4), 2.15 (1H, m, H3a), 1.99 (1H, t, 8.8 Hz, H5b), 1.69 (1H, m, H3b), 1.24 (3H, t, 7.1 Hz, Et), 0.98 (3H, d, 6.8 Hz, H6); ^{13}C NMR ($CDCl_3$, taken from 2D data at 500 MHz) δ 129.2 (CH, Bn), 128.3 (CH, Bn), 65.4 (CH, C2), 61.6 (CH_2, C5), 60.6 (CH_2, Et), 59.2 (CH_2, Bn), 37.8 (CH_2, C3), 31.4 (CH, C4), 18.9 (CH_3, C6), 14.4 (CH_3, Et); LRESIMS *m/z* = 248.1 [M+H]+ (248.2 calcd for $C_{15}H_{22}NO_2$).

Section 2.3.3

_N_-CBz-4-hydroxy-L-_trans_-proline ethyl ester, 2.49[7]

4-Hydroxy-L-*trans*-proline ethyl ester hydrochloride (1 g, 5.11 mmol) was dissolved in anhydrous MeOH (20 mL) and cooled to 0 ºC. TEA (2.15 mL, 15.4 mmol, 3.0 eq) was added followed by the dropwise addition of CBzCl (950 μL, 6.75 mmol, 1.3 eq). The reaction was warmed to room temperature and stirred for 16 hours. The solvent was removed *in vacuo*, ethyl acetate (30 mL) was added and then the mixture was washed with citric $acid_{(aq)}$ (5% w/v, 3 x 30 mL) and H_2O (2 x 20 mL). The organic phase was dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. The crude product was purified on silica using a stepwise gradient of 5 to 50% ethyl acetate in petroleum ether to give *N*-CBz-4-hydroxy-L-*trans*-proline ethyl ester (**2.49**, 1.46 g, 4.98 mmol, 98%) as a colourless oil (an inseparable ~1:1 mixture of *cis*- and *trans*-isomers around the carbamate was obtained).

R_f 0.27 (silica, 1:1 petroleum ether:ethyl acetate, visualization UV/PMA); $[\alpha]^{D}_{20}$ –55 (c 1.00, $CHCl_3$); IR (thin film) ν_{max} 1743, 1707, 1421, 1358, 1196 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.40-7.29 (5H, m, CBz), 5.16-5.03 (2H, m, CBz), 5.05 (1H, d, 12.7 Hz, O**H**), 4.51-4.46 (2H, m, H-4 and H-2), 4.21 (1H, q, 7.2 Hz, Et), 4.02 (1H, m, Et), 3.69-3.54 (2H, m, H-5), 2.31 (1H, m, H-3a), 2.10 (1H, m, H-3b), 1.27 (3/2H, t, 7.1 Hz, Et), 1.11 (3/2H, t, 7.1 Hz, Et); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.1 (C, ester C=O), 172.9 (C, ester C=O), 155.4 (C, CBz C=O), 155.0 (C, CBz C=O), 136.9 (C, CBz), 136.7 (C, CBz), 128.9 (CH, CBz), 128.8 (CH, CBz), 128.4 (CH, CBz), 128.3 (CH, CBz), 128.2 (CH, CBz), 70.6 (CH, C-4), 69.9 (CH, C-4), 67.7 (CH_2, CBz), 67.6 (CH_2, CBz), 61.7 (CH_2, Et), 61.6 (CH_2, Et), 58.4 (CH, C-2), 58.2 (CH, C-2), 55.6 (CH_2, C-5), 55.0 (CH_2, C-5), 39.6 (CH_2, C-3), 38.9 (CH_2, C-3), 14.5 (CH_3, Et), 14.4 (CH_3, Et); HRESIMS m/z = 294.1344 $[M+H]^+$ 1.0 ppm (294.1341 calcd for $C_{15}H_{20}NO_5$).

_N_-CBz-4-hydroxy-D-_cis_-proline ethyl ester, 2.50

Preparation as **2.49**, using 4-hydroxy-D-*cis*-proline ethyl ester hydrochloride as starting material. Product obtained as a colourless oil in approximately quantitative yield (an inseparable ~1:1 mixture of *cis*- and *trans*-isomers around the carbamate was obtained).

R_f 0.24 (silica, 1:1 petroleum ether:ethyl acetate, visualization UV/PMA); $[\alpha]^{D}_{20}$ +20 (c 0.10, $CHCl_3$); IR (thin film) ν_{max} 1748, 1705, 1418, 1351, 1201 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.38-7.30 (5H, m, CBz), 5.14 (2H, m, CBz), 4.43 (1H, d, 10.0 Hz, H-2), 4.38 (1H, m, H-4), 4.26 (1H, q, 7.0 Hz, Et), 4.08 (1H, m, Et), 3.78 (1/2H, d, 11.7 Hz, H-5a), 3.73 (1/2H, d, 11.7 Hz, H-5a), 3.64 (1/2H, dd, 12.2 Hz, 4.6 Hz, H-5b), 3.60 (1/2H, dd, 11.8 Hz, 4.5 Hz, H-5b), 2.34 (1H, m, H-3a), 2.12 (1H, m, H-3b), 1.31 (3/2H, t, 7.3 Hz, Et), 1.15 (3/2H, t, 7.2 Hz, Et); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.7 (C, ester C=O), 174.5 (C, ester C=O), 154.9 (C, CBz C=O), 154.2 (C, CBz C=O), 136.3 (C, CBz), 136.2 (C, CBz), 128.5 (CH, CBz), 128.4 (CH, CBz), 128.1 (CH, CBz), 127.9 (CH, CBz), 127.8 (CH, CBz), 71.3 (CH, C-4), 70.3 (CH, C-4), 67.3 (CH_2, CBz), 62.0 (CH_2, Et), 61.9 (CH_2, Et), 58.3 (CH, C-2), 57.9 (CH, C-2), 56.2 (CH_2, C-5), 55.9 (CH_2, C-5), 38.6 (CH_2, C-3), 37.7 (CH_2, C-3), 13.9 (CH_3, Et); HRESIMS m/z = 294.1329 $[M+H]^+$ 4.1 ppm (294.1341 calcd for $C_{15}H_{20}NO_5$).

_N_-CBz-4-keto-L-proline ethyl ester, 2.51

CrO_3 (6.30 g, 63.0 mmol, 13 eq) was dissolved in a mixture of pyridine (10.2 mL) and DCM (50 mL) and stirred at room temperature for 15 minutes. After this time *N*-CBz-*trans*-4-hydroxy-L-proline ethyl ester (**2.49**, 1.4 g, 4.77 mmol) dissolved in DCM (20 mL) was added to the mixture. The reaction was stirred at room temperature for 16 hours, then filtered through a silica pad using 50% ethyl acetate in petroleum ether (200 mL). After removal of the solvents *in vacuo*, the product (1.18 g, 4.05 mmol, 85%) was obtained as a colourless oil (an inseparable ~1:1 mixture of *cis*- and *trans*-isomers around the carbamate was obtained).

R_f 0.43 (silica, 2:1 petroleum ether:ethyl acetate, visualization UV/PMA); $[\alpha]^{D}_{20}$ –4 (c 1.00, $CHCl_3$); IR (thin film) ν_{max} 1767, 1743, 1713, 1416, 1192, 1158 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.38-7.32 (5H, m, CBz), 5.20 (2H, m, CBz), 4.85 (1/2H, d, 10.8 Hz, H-2), 4.81 (1/2H, d, 10.8 Hz, H-2) 4.22 (1H, q, 7.2 Hz, Et), 4.09 (1H, m, Et), 3.96 (2H, m, H-5), 2.95 (1H, m, H-3a), 2.60 (1H, dd, 18.8 Hz, 2.6 Hz, H-3b), 1.27 (3/2H, t, 7.2 Hz, Et), 1.16 (3/2H, t, 7.2 Hz, Et); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 207.8 (C, C-4), 207.1 (C, C-4), 171.3 (C, ester *C*=O), 154.9 (C, CBz *C*=O), 154.1 (C, CBz *C*=O), 135.9 (C, CBz), 129.0 (CH, CBz), 128.5 (CH, CBz), 128.3 (CH, CBz), 128.0 (CH, CBz), 67.7 (CH_2,

CBz), 61.8 (CH_2, Et), 56.0 (CH, C-2), 52.6 (CH_2, C-5), 52.4 (CH_2, C-5), 41.1 (CH_2, C-3), 40.6 (CH_2, C-3), 14.0 (CH_3, Et); HRESIMS *m/z* = 292.1191 $[M+H]^+$ 2.1 ppm (292.1185 calcd for $C_{15}H_{18}NO_5$).

OR

N-CBz-L-*trans*-4-hydroxyproline ethyl ester (**2.49**, 1 g, 3.41 mmol) and IBX (2.88 g, 10.2 mmol, 3.0 eq) were dissolved in DMSO (24 mL) at 70 ºC and stirred for 40 hours. After cooling the mixture to room temperature ethyl acetate (50 mL) was added and the mixture extracted with $NaHCO_{3\,(aq)}$ (1 M, 2 x 40 mL) and H_2O (1 x 40 mL). The organic phase was dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. The crude product was purified on silica using a stepwise gradient of 5 to 15% ethyl acetate in petroleum ether. The product (**2.51**, 360 mg, 1.24 mmol, 36%) was obtained as a colourless oil.

***N*-CBz-4-keto-D-proline ethyl ester, *ent*-2.51**

Preparation as **2.51** (CrO_3 method), using *N*-CBz-4-hydroxy-D-*cis*-proline ethyl ester, **2.50**. Product obtained as a colourless oil (*ent*-**2.51**, 87 %).

$[\alpha]^D_{20}$ +4 (c 1.00, $CHCl_3$).

***N*-CBz-L-4-exomethyleneproline ethyl ester, 2.52**

Wittig reaction (^{t}BuOK$_{(s)}$)

^{t}BuOK (73 mg, 0.65 mmol, 3 eq), was dissolved in anhydrous THF (3 mL) and $MePPh_3I$ (260 mg, 0.65 mmol, 3 eq) was added in one portion. The reaction was stirred for 15 minutes at room temperature. N-CBz-L-4-ketoproline ethyl ester (**2.51**, 62 mg, 0.21 mmol), dissolved in anhydrous THF (3 mL) was then added dropwise to the solution and the reaction was stirred for a further 20 hours at room temperature. After diluting the reaction with ethyl acetate (10 mL) the mixture was extracted with citric $acid_{(aq)}$ (5% w/v, 2 x 10 mL) and $NaHCO_{3(aq)}$ (1M, 2 x 10 mL). The organic phase was dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. The crude product was purified on silica using a stepwise gradient of 4 to 16% ethyl acetate in petroleum ether to give the product (**2.52**, 16 mg, 0.054 mmol, 26%) as a colourless oil.

$[\alpha]^D_{20}$ 0 (c 1.00, $CHCl_3$).

Wittig reaction (^{t}BuOK, 1M THF)

$MePPh_3I$ (390 mg, 0.97 mmol, 1.41 eq) was added in several portions over 5 minutes to a solution of ^{t}BuOK (960 μL of a 1M THF solution, 0.96 mmol, 1.39 eq, diluted in anhydrous THF, 2.5 mL) at room temperature and the mixture was then stirred for a further 15 minutes. *N*-CBz-L-4-ketoproline ethyl ester (**2.51**, 200 mg, 0.69 mmol) dissolved in anhydrous THF (1.3 mL) was then added dropwise to the mixture. The reaction was then stirred at room temperature for 2 hours. After diluting the reaction with ethyl acetate (10 mL) the mixture was extracted with citric $acid_{(aq)}$ (5% w/v, 2 x 10 mL) and $NaHCO_{3(aq)}$ (1M, 2 x 10 mL). The organic phase was dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. The crude product was purified on silica using a stepwise gradient of 4 to 12% ethyl acetate in petroleum ether to give the product (**2.52**, 22 mg, 0.076 mmol, 11%) as a colourless oil.

Petasis reaction

To *N*-CBz-4-keto-L-proline ethyl ester (**2.51**, 100 mg, 0.34 mmol), dissolved in anhydrous toluene (1 mL), was added a toluene solution of dimethyltitanocene[8] (**2.64**, see below, ~ 0.88 mmol in ~ 1 mL, 2.6 eq) and the reaction was heated to 90 ºC for 3 hours. After cooling to room temperature the mixture was added dropwise to stirred petroleum ether (50 mL), whereupon a yellow precipitate formed, and was stirred for a further 30 minutes. The precipitate was removed by filtration through a bed of celite and the solvents were removed *in vacuo*. The crude residue was purified by chromatography on silica using a stepwise gradient of 2 to 10% diethyl ether in petroleum ether to give the product (**2.52**, 54 mg, 0.19 mmol, 56%) as a colourless oil (an inseparable ~1:1 mixture of *cis*- and *trans*- isomers around the carbamate was obtained).

R_f 0.57 (silica, 2:1 petroleum ether:ethyl acetate, visualization UV/PMA); $[\alpha]^D_{20}$ −16 (c 1.00, $CHCl_3$); IR (thin film) ν_{max} 1747, 1714, 1417, 1360, 1196, 896 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.38-7.29 (5H, m, CBz), 5.14 (2H, m, H-6), 5.02 (2H, m, CBz), 4.55 (1/2H, dd, 9.6 Hz, 2.9 Hz, H-2), 4.50 (1/2H, dd, 9.6 Hz, 2.9 Hz, H-2), 4.17 (3H, m, H-5, Et), 4.07 (1H, m, Et), 2.98 (1H, m, H-3a), 2.64 (1H, br d, 16.1 Hz, H-3b), 1.26 (3/2H, t, 7.2 Hz, Et), 1.14 (3/2 H, t, 7.2 Hz, Et); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.1 (C, ester *C*=O), 172.0 (C, ester *C*=O), 154.9 (C, CBz *C*=O), 154.3 (C, CBz *C*=O), 142.9 (C, C-4), 142.0 (C, C-4), 136.5 (C, CBz), 136.4 (C, CBz), 128.8 (CH, CBz), 128.6 (CH,

CBz), 128.5 (CH, CBz), 128.4 (CH, CBz), 128.01 (CH, CBz), 127.97 (CH, CBz), 127.9 (CH, CBz), 127.8 (CH, CBz), 108.3 (CH_2, C-6), 108.2 (CH_2, C-6), 67.13 (CH_2, CBz), 67.07 (CH_2, CBz), 61.3 (CH_2, Et), 61.2 (CH_2, Et), 59.0 (CH, C-2), 58.9 (CH, C-2), 51.0 (CH_2, C-5), 50.5 (CH_2, C-5), 36.8 (CH_2, C-3), 36.0 (CH_2, C-3), 14.1 (CH_3, Et), 14.0 (CH_3, Et); HRESIMS *m/z* = 290.1404 $[M+H]^+$ 4.1 ppm (290.1392 calcd for $C_{16}H_{20}NO_4$).

_N_-CBz-4-exomethylene-D-proline ethyl ester, *ent*-2.52

Prepared by the Wittig reaction ($^tBuOK_{(s)}$), using *N*-CBz-4-keto-D-proline ethyl ester, ***ent*-2.51**. Product obtained as a colourless oil (***ent*-2.52**, 30%).

$[\alpha]^D_{20}$ 0 (c 1.00, $CHCl_3$).

Prepared by the Petasis reaction. Colourless oil obtained in 42% yield.

$[\alpha]^D_{20}$ +15 (c 1.00, $CHCl_3$).

_N_-CBz-4-methyl-L-*trans*-proline ethyl ester, 2.53 and intermediate olefin isomerisation product, 2.58

N-CBz-4-exomethylene-L-proline ethyl ester (**2.52**, 30 mg, 0.104 mmol) was dissolved in chloroform (1 mL) and Ir(cod)pyr(PCy_3)]PF_6 (Crabtree's catalyst, **2.43**, 3 mg, 0.004 mmol, 4 mol%) was added. The reaction was placed under an atmosphere of $H_{2(g)}$ by means of a balloon and stirred at room temperature for 5 days. The crude product was purified on silica using a stepwise gradient of 2 to 6% diethyl ether in petroleum ether to give the product (**2.53**, 25 mg, 0.087 mmol, 84%) as a colourless oil, plus isomerisation product (**2.58**, 3 mg, 0.010 mmol, 10%) as a colourless oil. Inseparable ~1:1 mixtures of *cis*- and *trans*-isomers around the carbamate were obtained for both products.

2.58 R_f 0.29 (silica, 4:1 petroleum ether:ethyl acetate, visualization UV/PMA); $[\alpha]^D_{20}$ -87 (c 0.30, $CHCl_3$); IR (thin film) ν_{max} 1751, 1709, 1427, 1203, 1183 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.38-7.30 (5H, m, CBz), 6.40 (1/2H, br s, H-5), 6.32 (1/2H, br s, H-5), 5.15 (2H, m, CBz), 4.70 (1/2H, dd, 12.0 Hz, 5.0 Hz, H-2), 4.64 (1/2H, dd, 12.0 Hz, 5.0 Hz, H-2), 4.23 (1H, q, 7.0 Hz, Et), 4.08 (1H, q, 7.1 Hz, Et), 2.99 (1H, m, H-3a), 2.53 (1H, m, H-3b), 1.70 (3/2H, br s, H-6), 1.68 (3/2H, br s, H-6), 1.28 (3/2H, t, 7.1 Hz, Et), 1.15 (3/2H, t, 7.1 Hz, Et); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 128.5 (CH, CBz), 128.0 (CH, CBz), 127.9 (CH, CBz), 124.3 (CH, C-5), 123.7 (CH, C-5), 67.3 (CH_2, CBz), 67.1 (CH_2, CBz), 61.4

(CH_2, Et), 58.7 (CH, C-2), 58.6 (CH, C-2), 39.6 (CH_2, C-3), 14.1 (CH_3, Et), 13.2 (CH_3, C-6), quaternary carbons not observed; HRESIMS *m/z* = 290.1404 $[M+H]^+$ 4.1 ppm (290.1392 calcd for $C_{16}H_{20}NO_4$).

2.53 R_f 0.24 (silica, 4:1 petroleum ether:ethyl acetate, visualization UV/PMA); $[\alpha]^D_{20}$ −43 (c 1.00, $CHCl_3$) (*cis*:*trans*, ~1:15); IR (thin film) ν_{max} 1747, 1710, 1417, 1357, 1194 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.38-7.28 (5H, m, CBz), 5.18-5.04 (2H, m, CBz), 4.41 (1/2H, dd, 9.0 Hz, 2.6 Hz, H-2), 4.36 (1/2H, dd, 9.0 Hz, 2.6 Hz, H-2), 4.19 (1H, q, 7.4 Hz, Et), 4.05 (1H, m, Et), 3.78 (1H, m, H-5a), 3.05 (1/2H, dd, 10.1 Hz, 8.5 Hz, H-5b), 2.99 (1/2H, dd, 10.1 Hz, 8.8 Hz, H-5b), 2.41 (1H, m, H-4), 2.09 (1H, m, H-3a), 1.84 (1H, m, H-3b), 1.26 (3/2H, t, 7.2 Hz, Et), 1.14 (3/2H, t, 7.2 Hz, Et), 1.05 (3/2H, d, 6.7 Hz, H-6), 1.03 (3/2H, d, 6.7 Hz, H-6); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.8 (C, ester *C*=O), 172.6 (C, ester *C*=O), 154.2 (C, CBz *C*=O), 136.7 (C, CBz), 128.4 (CH, CBz), 128.3 (CH, CBz), 127.91 (CH, CBz), 127.85 (CH, CBz), 127.8 (CH, CBz), 127.7 (CH, CBz), 66.94 (CH_2, CBz), 66.88 (CH_2, CBz), 61.1 (CH_2, Et), 61.0 (CH_2, Et), 59.5 (CH, C-2), 59.1 (CH, C-2), 53.6 (CH_2, C-5), 53.3 (CH_2, C-5), 38.5 (CH_2, C-3), 37.6 (CH_2, C-3), 32.0 (CH, C-4), 31.1 (CH, C-4), 17.33 (CH_3, C-6), 17.29 (CH_3, C-6), 14.14 (CH_3, Et), 14.05 (CH_3, Et); HRESIMS *m/z* = 292.1545 $[M+H]^+$ 1.4 ppm (292.1549 calcd for $C_{16}H_{22}NO_4$).

_N_-CBz-4-Methyl-D-_trans_-proline ethyl ester, _ent_-2.53:

Prepared as **2.53**, using *N*-CBz-4-exomethylene-D-proline ethyl ester, *ent*-**2.52**. Product obtained as a colourless oil (*ent*-**2.53**, 80%).

$[\alpha]^D_{20}$ +41 (c 0.31, $CHCl_3$) (*cis*:*trans*, ~1:15).

4-Methyl-L-_cis_-proline ethyl ester, 2.54

N-CBz-4-Exomethylene-L-proline ethyl ester (**2.52**, 15 mg, 0.052 mmol) was dissolved in DCM (1 mL) and Pd/C (10%, 1.5 mg) added. The reaction was placed under an atmosphere of $H_{2(g)}$ by means of a balloon and stirred at room temperature for 16 hours. The catalyst was removed by filtration through a pad of celite using DCM (15 mL) and the solvent removed *in vacuo* to give the product (**2.54**, 5.5 mg, 0.035 mmol, 67%) as a colourless oil. $[\alpha]^D_{20}$ −36 (c 0.31, $CHCl_3$) (*cis*:*trans*, ~7:1); IR (thin film) ν_{max} 3418, 1743, 1263, 1230 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 4.47 (1H, t, 8.8 Hz, H-2), 4.30 (2H, q,

7.1 Hz, Et), 3.68 (1H, m, H-5a), 2.98 (1H, br t, 10.5 Hz, H-5b), 2.63-2.56 (2H, m, H-3a and H-4), 1.66 (1H, m, H-3b), 1.32 (3H, t, 7.1 Hz, Et), 1.13 (3H, d, 6.4 Hz, H-6); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.1 (C, ester *C*=O), 62.9 (CH_2, Et), 59.0 (CH, C-2), 52.3 (CH_2, C-5), 36.8 (CH_2, C-3), 33.2 (CH, C-4), 16.4 (CH_3, C-6), 14.0 (CH_3, Et); HRESIMS *m/z* = 158.1183 $[M+H]^+$ 1.3 ppm (158.1181 calcd for $C_8H_{16}NO_2$).

4-Methyl-D-*cis*-proline ethyl ester, ***ent*****-2.54**

Prepared as **2.54**, using *N*-CBz-4-exomethylene-D-proline ethyl ester, ***ent*****-2.52**. Product obtained as a colourless oil (***ent*****-2.54**, ~100%).

$[\alpha]^{D}_{20}$ +39 (c 0.30, $CHCl_3$) (*cis*:*trans*, ~7:1).

4-Methyl-L-*trans*-proline ethyl ester, 2.55

N-CBz-4-Methyl-L-*trans*-proline ethyl ester (**2.53**, ~2 mg, ~0.007 mmol) was dissolved in DCM (1 mL) and Pd/C was added (10 %, ~0.1 mg). The reaction was placed under $H_{2(g)}$ at atmospheric pressure by means of a balloon and stirred at room temperature for 16 hours. The catalyst was removed by filtration through a pad of celite using DCM (3 mL) and the solvent removed *in vacuo* to give the product as a colourless oil (**2.55**, ~ 100%).

^{1}H NMR (CD_3OD, 500 MHz) δ 4.52 (1H, dd, 9.1 Hz, 5.1 Hz, H1), 4.31 (2H, q, 7.1 Hz, Et), 3.55 (1H, dd, 11.3 Hz, 7.5 Hz, H5a), 2.90 (1H, m, H5b), 2.46-2.32 (2H, m, H3a and H4), 2.02 (1H, m, H3b), 1.32 (3H, t, 7.1 Hz, Et), 1.14 (3H, d, 6.4 Hz, H6).

4-Methyl-D-*trans*-proline ethyl ester, ***ent*****-2.55**

Prepared as **2.55**, using *N*-CBz-4-Methyl-D-*trans*-proline ethyl ester, ***ent*****-2.53**. Product obtained as a colourless oil (***ent*****-2.55**, ~ 100%).

4-Methyl-L-*trans*-proline hydrochloride, 2.56

N-CBz-4-Methyl-L-*trans*-proline ethyl ester (**2.53**, 4 mg, 0.014 mmol) was dissolved in $HCl_{(aq)}$ (6 M, 300 μL), sealed in a thick-walled glass tube and heated to 70 ºC for 6 hours. After cooling to room temperature, the reaction was extracted with ethyl acetate (2 x 300 μL) and the $HCl_{(aq)}$ removed by evaporation under a stream of $N_{2(g)}$ to give the product (2.3 mg, 0.014 mmol, ~100%) as an white amorphous solid.

$[\alpha]^{D}_{20}$ –33 (c 0.21, H_2O) (*cis*:*trans*, ~1:15) (lit. free amine –56.6º, c 1.03, H_2O; –23.9º, c 1.54, 3N HCl);[9] IR (diffuse reflectance) ν_{max} 1736, 1456, 1358, 1265, 1211 cm^{-1}; ^1H NMR (D_2O, 500 MHz) δ 4.38 (1H, dd, 9.6 Hz, 5.1 Hz, H-2), 3.49 (1H, dd, 11.6 Hz, 7.2 Hz, H-5a), 2.83 (1H, dd, 11.5 Hz, 8.6 Hz, H-5b), 2.38-2.25 (2H, m, H-3a and H-4), 1.94 (1H, dt, 13.2 Hz, 9.0 Hz, H-3b), 0.99 (3H, d, 6.6 Hz, H-6); ^{13}C NMR (D_2O, 75 MHz) δ 172.8 (C, *C*=O), 59.9 (CH, C-2), 52.9 (CH_2, C-5), 36.4 (CH_2, C-3), 32.3 (CH, C-4), 16.6 (CH_3, C-6); HRESIMS *m/z* = 130.0862 $[M+H]^+$ 4.6 ppm (130.0868 calcd for $C_6H_{12}NO_2$).

4-Methyl-D-*trans*-proline hydrochloride, *ent*-2.56

As described above, using 4-methyl-D- *trans*-proline ethyl ester (*ent*-**2.53**). Amorphous white solid obtained in 80% yield.

$[\alpha]^{D}_{20}$ +31 (c 0.04, H_2O) (*cis*:*trans*, ~1:15).

4-Methyl-L-*cis*-proline hydrochloride, 2.57

As described above, using 4-methyl-L-*cis*-proline ethyl ester (**2.54**). Amorphous white solid obtained in approximately quantitative yield.

$[\alpha]^{D}_{20}$ –41 (c 0.07, H_2O) (*cis*:*trans*, ~7:1); IR (diffuse reflectance) ν_{max} 3100-2900, 1738, 1455, 1405, 1213 cm^{-1}; ^1H NMR (D_2O, 500 MHz) δ 4.30 (1H, dd, 8.2 Hz, 9.8 Hz, H-2), 3.43 (1H, dd, 11.4 Hz, 7.4 Hz, H-5a), 2.87 (1H, br t, 10.6 Hz, H-5b), 2.52 (1H, dt, 13.1 Hz, 7.6 Hz, H-3a), 2.38 (1H, m, H-4), 1.64 (1H, dt, 13.1 Hz, 9.8 Hz, H-3b), 0.99 (3H, d, 6.7 Hz, H-6); ^{13}C NMR (D_2O, 75 MHz) δ 173.0 (C, *C*=O), 60.7 (CH, C-2), 52.7 (CH_2, C-5), 36.9 (CH_2, C-3), 33.7 (CH, C-4), 16.4 (CH_3, C-6); HRESIMS *m/z* = 130.0862 $[M+H]^+$ 4.6 ppm (130.0868 calcd for $C_6H_{12}NO_2$).

4-Methyl-D-*cis*-proline hydrochloride, *ent*-2.57

As described above, using 4-methyl-D-*cis*-proline ethyl ester. Amorphous white solid obtained in 87% yield.

$[\alpha]^{D}_{20}$ +31 (c 0.12, H_2O) (*cis*:*trans*, ~7:1) (lit. free amine +85.2º, c 1.68, H_2O; +47.9º, c 1.28, 3N HCl).[9]

Dimethyltitanocene (Petasis reagent), 2.64 [8]

Titanocene dichloride (Cp_2TiCl_2, 220 mg, 0.88 mmol) was dissolved in dry toluene (2 mL) and cooled in an ice-bath. MeLi (1.6 M in diethyl ether, 1.27 mL, 2.03 mmol, 2.3 eq) was added dropwise and the reaction stirred for 1 hour. The mixture was poured slowly into a cooled solution of NH_4Cl (6% w/v, 2 mL), the organic phase separated, washed with further NH_4Cl solution (2 mL), dried over $MgSO_4$, filtered and the solvent reduced (~ 1 mL) *in vacuo* to give an orange solution of product.

Epimerisation of 4-hydroxy-L-*trans*-proline to give 4-hydroxy-D-*cis*-proline [10]

Acetic anhydride (400 μL, 4.2 mmol, 5.5 eq) and glacial acetic acid (1.27 mL, 22 mmol, 29 eq) were warmed to 50 ºC and 4-hydroxy-L-*trans*-proline (100 mg, 0.76 mmol) was added. The reaction was then heated to reflux for 5.5 hours. The reaction was cooled to room temperature and the solvents removed *in vacuo*. The crude residue was taken up in 2M HCl(aq) (1.38 mL) and refluxed for a further 3 hours. A spatula of activated carbon was added to the mixture whilst hot, and after cooling to room temperature the solution was filtered though a bed of celite (washed though with extra 2 mL of deionised H_2O). The solvent was removed *in vacuo* to give the product as a white amorphous solid.

Marfey's derivatisation general procedure [11]

A 1% (w/v) solution (100 μL) of FDAA (Marfey's reagent, N^{α}-(2,4-dinitro-5-fluorophenyl)-L-alaninamide) in acetone was added to an aliquot (50 μL) of a 50 mM solution of each amino acid. After addition of $NaHCO_3$ solution (1 M; 20 μL), the mixture was incubated (1 hour at 40 ºC). The reaction was stopped by addition of HCl (2 M, 10 μL), the solvents were evaporated to dryness, and the residue was redissolved in MeOH-H_2O (1:1; 1 mL). An aliquot of each of these solutions (10 μL) was analyzed by HPLC (see below).

HPLC analysis of Marfey's derivatives

All Marfey's derivatives were analysed on a Prodigy C_{18}, 250 × 4.6 mm, 5 μm column. The elution conditions used are described below.

4-Methylprolines 2.56, *ent*-**2.56**, **2.57** and *ent*-**2.57**:

solvents: A: H_2O + 0.05% TFA, B: MeOH; 0 min 45% B, 30 min 65% B; 25 ºC; 1 mL.min^{-1}; detection at 330 nm. R_t: **2.56**, 18.26 min; *ent*-**2.56**, 19.25 min; **2.57**, 16.62 min; *ent*-**2.57**, 18.48 min.

4-Methyl-L-*cis*-proline ethyl ester, 2.54:

solvents: A: H_2O + 0.05% TFA, B: MeCN; linear gradient: 0 min 35% B, 30 min 45% B; 25 ºC; 1 mL min^{-1}; detection at 330 nm. R_t: **L-*trans***, 23.87 min; **L -*cis***, 26.36 min.

4-Hydroxy-L-*trans*-proline epimerisation product and commercial 4-hydroxyproline diastereoisomers:

solvents: A: H_2O + 0.05% TFA, B: MeCN; linear gradient: 0 min 20% B, 30 min 35% B, 31 min 100% B; 25 ºC; 1 mL min^{-1} detection at 330 nm. R_t: **L-*trans***, 12.20 min; **L-*cis***, 13.29 min; **D-*trans***, 10.77 min; **D-*cis***, 12.55 min.

solvents: A: H_2O + 0.05% TFA, B: MeCN; isocatic: 30 min 18% B, 31 min 100% B; 25 ºC; 1 mL min^{-1}; detection at 330 nm. R_t: **L-*trans***, 19.57 min; **D-*cis***, 21.93 min.

Elution conditions and retention times of all other amino acids (except those analysed on a Shimadzu HPLC system) are given in **Table 2.3**.

Eluent A corresponds to the following elution system: solvents: A: H_2O + 0.05 % TFA, B: MeCN; linear gradient: 0 min 35 % B, 30 min 45 % B; 25 º C; 1 mL min^{-1}; detection at 330 nm.

Eluent B corresponds to the following elution system: solvents: A: H_2O + 0.05 % TFA, B: MeOH; linear gradient: 0 min 45 % B, 30 min 65 % B; 24 º C; 1 mL min^{-1}; detection at 330 nm.

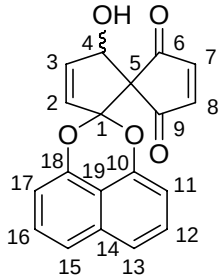
Elution conditions for those amino acids analysed on a Shimadzu HPLC are below:

Shimadzu eluent - solvents: A: H_2O + 0.05% TFA, B: MeCN; 0 min 20% B, linear gradient: 2 min 20% B, 32 min 45% B, 35 min 100% B; 40 ºC; 1 mL min^{-1}; detection at 330 nm.

5.3 Work described in Chapter 3

Unless otherwise stated, all HPLC analysis of reactions described below was carried out using on a Prodigy C_{18}, 250 × 4.6 mm, 5 μm column. The elution conditions used were as follows; solvents: A: H_2O + 0.05% TFA, B: MeCN; 0 min10%, linear gradient: 2 min 10% B, 14 min 75% B, linear gradient: 24 min 75 % B, 26 min 100% B; 40 ºC; 1 mL min^{-1}.

The carbon skeleton of all synthetic analogues of *spiro*-mamakone A, as well as those derivatives corresponding to the carbon skeleton of the northern hemisphere of *spiro*-mamakone A, are numbered here in accordance with that reported for *spiro*-mamakone A:[12]



The numbering of all other carbon skeleton systems are described below, as appropriate.

Section 3.2.1

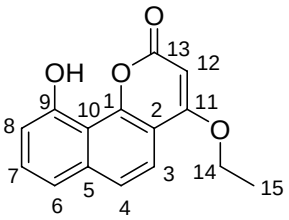
1,8-Dihydroxynaphthalene (DHN)[13]

1,8-Naphthalenesultone (6 g, 29.1 mmol) and potassium hydroxide (24.4 g, 438 mmol, 15 eq) were fused at 220 ºC until they formed a tarry black oil. Heating was continued for around 5 minutes after which time the reaction was allowed to cool to room temperature. Aqueous HCl (4M) was added until the reaction had been acidified and the mixture was extracted with ethyl acetate (5 x 100 mL). The combined organic phase was dried over $MgSO_4$, filtered and the solvent was removed *in vacuo*. The crude product was purified by silica chromatography using a step-wise gradient of 7 to 15% diethyl ether in petroleum ether. This gave 3.44 g (21.5 mmol, 74%) of product as a white solid;

mp 139-141 ºC (recrystallised from petroleum ether, lit 141-142 ºC);[13] R_f 0.32 (silica, 4:1 petroleum ether:ethyl acetate, visualization UV/PMA); IR (diffuse reflectance) ν_{max} 3300-3100, 1612, 1466, 1412, 1280, 1234, 1034 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.59 (2H, s, O**H**), 7.36 (2H, d, 8.2 Hz, H4/6), 7.29 (2H, dd, 8.2, 7.5 Hz, H3/7), 6.78 (2H, d, 7.5 Hz, H2/8); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.7, 137.0, 126.7, 120.5, 109.3, C9 not observed; HRESIMS *m/z* = 159.0440 $[M-H]^-$ 3.8 ppm (159.0446 calcd for $C_{10}H_7O_2$).

4-Ethoxy-10-hydroxy-2*H*-benzo[*h*]chromen-2-one, 3.32

DHN (60 mg, 0.38 mmol) was dissolved in ethyl-3,3-ethoxyacrylate (**3.28**, 210 µL, 1.14 mmol, 3 eq) and pTSA (2 mg, 0.01mmol, 3 mole%) was added. The reaction was heated to 85 ºC with stirring for 3 hours, then cooled to room temperature. The crude product was purified by silica chromatography using a step-wise gradient of 0 to 20% ethyl acetate in petroleum ether. This gave the product (**3.32**, 31 mg, 0.12 mmol, 32%) as a tan solid.



mp 191-193 ºC (recrystallised from petroleum ether); R_f 0.20 (silica, 4:1 petroleum ether:ethyl acetate, visualization UV/PMA); IR (diffuse reflectance) ν_{max} 3300-3000, 1680, 1626, 1611, 1490, 1343 1269, 1224 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.84 (1H, br s, OH), 7.76 (1H, d, 9.2 Hz, H3 or H4), 7.64 (1H, d, 9.1 Hz, H3 or H4), 7.54 (1H, t, 8.3 Hz, H7), 7.40 (1H, d, 8.0 Hz, H6), 7.10 (1H, d, 7.8 Hz, H8), 5.72 (1H, s, H12), 4.26 (2H, q, 7.0 Hz, H14), 1.59 (3H, t, 7.0 Hz, H15); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.7 (C, C11 or C13), 160.7 (C, C11 or C13), 154.5 (C, C9), 152.0 (C, C1), 137.2 (C, C5), 130.3 (CH, C7), 124.7 (CH, C3 or C4), 119.4 (CH, C6), 118.7 (CH, C3 or C4), 113.1 (C, C8), 112.2 (C, C2 or C10), 110.2 (C, C2 or C10), 88.5 (CH, C12), 65.5 (CH_2, C14), 14.1 (CH_3, C15); LREIMS $[M]^{+\cdot}$ 256, 228, 186.

Section 3.2.2

2,2-Diallylcyclopentane-1,3-dione, 3.26 [14]

1,3-Cyclopentadione (1 g, 10.2 mmol) was suspended in benzene (20 mL), allyl alcohol (1.4 mL, 20.5 mmol, 2.01 eq), $Pd_2(dba)_3$ (230 mg, 0.25 mmol, 0.025 eq) and triphenylphosphite (530 μL, 2.02 mmol, 0.20 eq) were added and the reaction was refluxed for 3 hours. The formation of H_2O was observed during the reaction. Upon cooling, the solvent was removed *in vacuo*. This crude material could then be used for ring-closing metathesis. Purification was possible by silica chromatography using a step-wise gradient of 2 to 8% diethyl ether in petroleum ether. This gave the product (**3.26**) as a volatile colourless oil in around 85%;

R_f 0.35 (silica, 4:1 petroleum ether:ethyl acetate, visualisation PMA); IR (thin film) ν_{max} 1728, 1420, 1196 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.56 (2H, m), 5.07 (2H, br d, 4.4 Hz), 5.05 (2H, br s), 2.62 (4H, s), 2.37 (4H, d, 7.6 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 216.4, 131.3, 120.0, 61.3, 39.4, 36.4; HREIMS *m*/*z* = 178.0994 $[M]^+$ 0.1 ppm (178.0994 calcd for $C_{11}H_{14}O_2$).

***spiro*-Nona-7-ene-1,4-dione, 3.27** [15]

Crude 2,2-diallylcyclopentane-1,3-dione (**3.26**, approx. 1.8 g) was dissolved in anhydrous DCM (30 mL) under an inert atmosphere. Grubbs' 2nd generation catalyst was added (350 mg, 0.41 mmol, approx. 4 mol%) and the reaction was stirred under an equilibrating inert atmosphere for 18 hours. Purification was carried out by silica chromatography using a step-wise gradient of 20 to 30% diethyl ether in petroleum ether. This gave 1.48 g (**3.27**, 9.88 mmol, 97% over two steps) of a slightly yellow solid;

mp 91-93 ºC (recrystallised from diethyl ether, lit. 89-90 ºC);[15] R_f 0.18 (silica, 4:1 petroleum ether:ethyl acetate, visualization PMA); IR (diffuse reflectance) ν_{max} 1751, 1713, 1443, 1273, 1227, 1111 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.62 (2H, br s), 2.83 (4H, s), 2.67 (4H, br s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 214.4, 127.4, 61.3, 41.1, 34.9; HRCIMS *m*/*z* = 150.0684 $[M]^+$ 2.2 ppm (150.0681 calcd for $C_9H_{10}O_2$).

Thermal degradation of 3.27 to *spiro*-nona-6-ene-1,4-dione, 3.36

Ketone **3.26** was heated to 80 ºC at atmospheric pressure in a glass finger containing a cooling tube (H_2O). Sublimation was observed and the crystalline solid collected. ^{1}H NMR spectroscopy showed considerable quantities of the thermal degradation product **3.36** as well as **3.27** (ratio of ~3:1).

3.36 ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.12 (1H, dt, 5.6 Hz, 2.3 Hz, H6 or H7), 5.38 (1H, dt, 5.6 Hz, 2.3 Hz, H6 or H7), 2.95-2.76 (4H, m, H2 and H3), 2.61 (2H, m, H8), 2.18 (2H, t, 7.4 Hz, H9); ^{13}C NMR ($CDCl_3$, HSQC data at 500 MHz) δ 127.0 (CH, C6 or C7), 33.0 (CH_2, C8), 30.6 (CH_2, C9), no other carbons observed.

***spiro*-Nona-6-ene-1,4-dione-1,1-[1,8-dihydroxynaphthalene]-acetal, 3.37**

DHN (430 mg, 2.68 mmol, 2.0 eq) and **3.27** (200 mg, 1.33 mmol) were dissolved in anhydrous toluene and triflic acid (30 µl, 0.34 mmol, 0.25 eq) was added. The reaction was heated under Dean-Stark refluxing conditions at 125 ºC for 16 hours. The reaction was cooled to room temperature, extracted with $NaHCO_{3(aq)}$ (1M, 3 x 4 mL), dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. The crude residue was purified by chromatography on Florisil using 0 to 1% diethyl ether in petroleum ether to give a mixture of **3.35** (for full characterisation, *vide infra*) and **3.37** in approximately 1:1 ratio (82 mg, 0.28 mmol, 21%).

R_f 0.45 (silica, 4:1 petroleum ether:ethyl acetate, visualization PMA); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.48 (2H, d, 8.2 Hz), 7.41 (2H, dd, 8.1 Hz, 7.5 Hz), 6.90 (2H, 7.5 Hz), 6.13 (1H, dt, 5.7 Hz, 2.4 Hz), 5.64 (1H, dt, 5.7 Hz, 2.2 Hz), 2.58-2.43 (4H, m), 2.30-2.09 (4H, m); ^{13}C NMR ($CDCl_3$, 2D NMR data, 500 MHz) δ 215.2 (C, C4), 147.8 (C, C10 and C19), 137.6 (CH, C6 or C7), 134.3 (C, C14), 127.5 (CH, C12 and C16), 126.9 (CH, C6 or C7), 120.8 (CH, C13 and C15), 114.2 (C, C19), 109.6 (CH, C11 and C17), 108.2 (C, C1), 71.8 (C, C5), 34.7 (CH_2, C2, C3, C8 or C9), 32.6 (CH_2, C2, C3, C8 or C9), 29.3 (CH_2, C2, C3, C8 or C9), 28.6 (CH_2, C2, C3, C8 or C9).

Cyclopentanone-1,1-dimethoxyacetal, 3.40

Cyclopentanone (1 mL, 12.5 mmol) was dissolved in dimethoxypropane (15 mL) and pTSA (60 mg, 0.32 mmol, 0.026 eq) was added. The reaction was refluxed for 16 hours, then cooled. Diethyl ether (20 mL) was added, the mixture washed with $NaHCO_{3\,(aq)}$ (1M, 3 x 20 mL), dried over $MgSO_4$, filtered and the solvents were removed *in vacuo*. This gave the product as a colourless oil which was used without further purification.
^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.03 (6H, s, OC**H**$_3$), 1.56 (4H, br dd, 7.3Hz, H2/5), 1.46 (4H, br dd, 7.3 Hz, H3/4); ^{13}C NMR ($CDCl_3$, from HSQC data at 500 MHz) δ 49.5 (CH_3, O**C**H_3), 34.4 (CH_2, C2/5), 23.3 (CH_2, C3/4).

Cyclopentanone-1,1-[1,8-dihydroxynaphthalene]-acetal, 3.41

Cyclopentanone-1,1-dimethoxyacetal (**3.40**, 20 mg, 0.13 mmol) and 1,8-dihydroxynaphthalene (20 mg, 0.15 mmol, 1.2 eq) were dissolved in dried, degassed toluene (0.5 mL) and triflic acid (3 μL, 0.011 mmol, 0.08 eq) was added, whereupon the mixture became dark. The reaction was stirred at room temperature for 20 hours, then diethyl ether (1 mL) was added and the reaction was washed with $NaHCO_{3\,(aq)}$ (1M, 3 x 1 mL), dried over $MgSO_4$, filtered and the solvents were removed *in vacuo*. The crude product was purified by chromatography on silica using petroleum ether to elute the product (16 mg) as a colourless oil which contained **3.41** contaminated with acetonide **3.39** in a ratio of ~1:1 (**3.41** ~8 mg, 0.035 mmol, 28%).
R_f 0.76 (4:1 petroleum ether:ethyl acetate, visualization UV/PMA); IR (thin film) ν_{max} 1605, 1412, 1381, 1273 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.43 (2H, dd, 8.3 Hz, 0.8 Hz, H9/H11), 7.37 (2H, dd, 7.4 Hz, 8.3 Hz, H8/12), 6.86 (2H, dd, 7.4 Hz, 0.8 Hz, H7/13), 2.05 (4 H, m, H2/5), 1.85 (4H, m, H3/4); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 148.9 (C, C6/14), 134.4 (C, C10), 127.1 (CH, C8/12), 120.1 (CH, C9/11), 114.3 (C, C15), 112.7 (C, C1), 109.0 (CH, C7/13), 37.0 (CH_2, C2/5), 23.8 (CH_2, C3/4); HREIMS *m/z* = 226.0992 $[M]^{+\cdot}$ 0.8 ppm (226.0994 calcd for $C_{15}H_{14}O_2$).

spiro-Nona-7-ene-1,4-dione-1,1-dimethoxyacetal, 3.38

spiro-Nona-7-ene-1,4-dione (**3.26**, 100 mg, 0.51 mmol) was dissolved in dimethoxypropane (2 mL) and pTSA (4 mg, 0.02 mmol) was added. The reaction was refluxed for 16 hours, then cooled to room temperature. Diethyl ether (3 mL) was added and the mixture was washed with $NaHCO_3$ (aq) (1M, 2 x 2 mL). The organic phase was dried over $MgSO_4$, filtered and the solvents removed *in vacuo*. The crude product which was obtained as a yellow oil (approximately quantitative yield as judged by NMR spectroscopy) was used in subsequent steps without further purification.

^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.62 (2H, br s, H7/8), 3.25 (6H, s, OC**H**$_3$), 2.32 (2H, t, 8 Hz, H3), 2.06 (2H, t, 8 Hz, H2); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 217.3 (C1), 128.3 (C7/8), 107.5 (C4), 61.5 (C5), 49.4 (O**C**H$_3$), 38.0 (C6/9), 33.4 (C3), 26.7 (C2).

Preparation of s*piro*-nona-7-ene-1,4-dione-1,1-[1,8-dihydroxynaphthalene]-acetal (or 4-keto-2,3-dihydro-6,9-deoxy-*spiro*-mamakone A), 3.35, via transacetalisation of DHN and 3.38

DHN (50 mg, 0.31 mmol) and *spiro*-nona-7-ene-1,4-dione-1,1-dimethoxyacetal (**3.38**, ~ 0.33 mmol, 1 eq) were dissolved in anhydrous toluene (1 mL) and triflic acid (12 μL, 0.14 mmol, 0.45 eq) was added. The reaction mixture became a dark brown upon addition of the acid and was stirred at 45 ºC for 16 hours. The reaction mixture was then diluted with diethyl ether (2 mL) and washed with $NaHCO_{3\,(aq)}$ (1M, 2 x 1 mL). The organic phase was dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. The crude residue was purified on silica using a stepwise gradient of petroleum ether to 20% diethyl ether in petroleum ether. This gave the product as an off-white solid (**3.35**, 22 mg, 0.075 mmol, 24%);

mp 150-151 ºC (recrystallised from diethyl ether); R_f 0.46 (silica, 4:1 petroleum ether:ethyl acetate, visualization UV/PMA); IR (diffuse reflectance) ν_{max} 1744, 1611, 1414, 1275, 1248 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.49 (2H, dd, 8.4 Hz, 0.7 Hz, H13/15), 7.41 (2H, dd, 8.3 Hz, 7.5 Hz, H12/16), 6.91 (2H, dd, 7.5 Hz, 0.7 Hz, H11/17), 5.70 (2H, br m, H7/8), 3.05 (2H, br d, 14 Hz, H6/9), 2.61 (2H, br d, 14 Hz, H6/9), 2.53 (2H, dd, 8.2 Hz, 7.7 Hz, H2 or H3), 2.17 (2H, dd, 8.2 Hz, 7.7 Hz, H2 or H3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 214.3 (C, C4), 147.5 (C, C10/18), 134.2 (C, C14), 128.1 (CH, C7/8),

127.3 (CH, C12/16), 120.6 (CH, C13/15), 113.9 (C, C19), 109.3 (CH, C11/17), 108.1 (C, C1), 62.0 (C, C5), 36.4 (CH_2, C6/9), 33.7 (CH_2, C2 or C3), 28.1 (CH_2, C2 or C3); HRESIMS *m/z* = 293.1187 $[M+H]^+$ 3 ppm (293.1178 calcd for $C_{19}H_{17}O_3$).

1,8-Dihydroxynaphthalene acetonide, 3.39

1,8-Dihydroxynaphthalene (640 mg, 4.0 mmol) was dissolved in dimethoxypropane (10 mL) and pTSA (10 mg, 0.06 mmol) was added. The reaction was refluxed for 16 hours, then cooled to room temperature. Diethyl ether (10 mL) was added and the mixture was washed with $NaHCO_3$ (aq) (1M, 2 x 10 mL). The organic phase was dried over $MgSO_4$ and filtered. The crude residue was passed through a short silica column with petroleum ether then the solvent was removed *in vacuo* to give a yellow solid/oil. Removal of the yellow oily impurity could be achieved by passing the residue through a short C_{18} reverse phase pad with a solution of 60% MeCN in H_2O. Removal of MeCN *in vacuo* yielded an aqueous suspension which was extracted exhaustively using diethyl ether. The combined organic phases were dried over $MgSO_4$, filtered and the solvent removed *in vacuo* to yield the product (**3.39**, 590 mg, 2.95 mmol, 74%) as a white crystalline solid. mp 61-64 ºC (recrystallised from petroleum ether); R_f 0.75 (silica, 4:1 petroleum ether:ethyl acetate, visualization UV/PMA); IR (thin film) ν_{max} 1608, 1411, 1385, 1375, 1281, 1265 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.4 (4H, m, H3/4/6/7), 6.86 (2H, dd, 6.8 Hz, 1.4 Hz, H2/8), 1.66 (6H, s, H12/13); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 148.0 (C1/9), 134.2 (C5), 127.3 (C3/7), 120.0 (C4/6), 113.5 (C10), 108.7 (C2/8), 101.7 (C11), 25.2 (C12/13); HREIMS *m/z* = 200.0835 $[M]^{+\cdot}$ 1.0 ppm (200.0837 calcd for $C_{13}H_{12}O_2$).

Preparation of s*piro*-nona-7-ene-1,4-dione-1,1-[1,8-dihydroxynaphthalene]-acetal (or 4-keto-2,3-dihydro-6,9-deoxy-*spiro*-mamakone A), 3.35, via transacetalisation of 3.39 and 3.27

1,8-Dihydroxynaphthalene acetonide (**3.39**, 900 mg, 4.50 mmol, 1.7 eq) and s*piro*-nona-7-ene-1,4-dione (**3.27**, 400 mg, 2.66 mmol) were dissolved in chloroform (6 mL) and triflic acid (40 μL, 0.45 mmol, 0.17 eq) was added. The reaction mixture became a dark brown upon addition of the acid and was stirred at 45 ºC for 48 hours. The reaction mixture was then diluted with diethyl ether (2 mL) and washed with $NaHCO_{3\,(aq)}$ (1M, 2 x

1 mL). The organic phase was dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. The crude residue was purified on Florisil solid phase using a stepwise gradient of 0 to 20% diethyl ether in petroleum ether. This gave the product as an off-white solid (**3.35**, 120 mg, 0.41 mmol, 15%).

Section 3.2.3

spiro-Nona-2,7-diene-1,4-dione, 3.44

spiro-Nona-7-ene-1,4-dione (**3.27**, 200 mg, 1.33 mmol) was dissolved in MeOH (20 mL) and $CuBr_2$ (630 mg, 2.82 mmol, 2.1 eq) was added. The reaction was refluxed for 2 hours, then cooled to room temperature. After removal of solvent *in vacuo*, the crude residue was purified on silica using a stepwise gradient of 5% to 20% diethyl ether in petroleum ether. This gave the product as a faintly yellow crystalline solid (**3.44**, 190 mg, 1.28 mmol, 96%).

mp 33-35 ºC; R_f 0.20 (4:1 petroleum ether:ethyl acetate, visualization UV/PMA); IR (diffuse reflectance) ν_{max} 1705, 1327, 1265 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.30 (2H, s, H2/3), 5.69 (2H, br s, H7/8), 2.64 (4H, br s, H6/9); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 207.0 (C1/4), 149.3 (C2/3), 128.5 (C7/8), 54.3 (C5), 41.0 (C6/9); HREIMS *m/z* = 148.0521 $[M]^{+\cdot}$ 2.5 ppm (148.0524 calcd for $C_9H_8O_2$).

Mono-reduction of 3.44 to give 4-hydroxy-*spiro*-nona-2,7-diene-1-one, 3.45 and 4-hydroxy-*spiro*-nona-7-ene-1-dione, 3.46

spiro-Nona-2,7-diene-1,4-dione (**3.44**, 230 mg, 1.6 mmol, 2 eq) was dissolved in anhydrous MeOH (3 mL) and cooled to – 78 ºC using an acetone/dry ice bath. $NaBH_4$ (30 mg, 0.8 mmol) was added and the reaction stirred at – 78 ºC for 1 hour. Citric $acid_{(aq)}$ (5 % w/v, 6 mL) was added and after warming to room temperature, the reaction was extracted with diethyl ether (10 mL). The organic phase was then washed with $NaHCO_{3(aq)}$ (1M, 5 mL), dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. The crude material was purified by chromatography on silica using a stepwise gradient of 15 to 25% diethyl ether in petroleum ether. This gave the product (**3.45**, 50 mg, 0.33

mmol, 42%) as a colourless oil, with 4-hydroxy-s*piro*-nona-7-ene-1-dione (**3.46**, 36 mg, 0.24 mmol, 30%) and starting material (**3.44**, 22 mg, 0.15 mmol, 19%) also recovered.
3.45: R_f 0.22 (2:1 petroleum ether:ethyl acetate, visualization UV/PMA); IR (thin film) ν_{max} 3419, 1699, 1338, 1272, 1107 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.51 (1H, dd, 5.9 Hz, 2.3 Hz, H3), 6.29 (1H, dd, 5.9 Hz, 1.3 Hz, H2), 5.70 (2H, m, H7/8), 4.68 (1H, br s, H4), 2.79 (2H, m, H6 or H9), 2.46 (1H, m, H6 or H9), 2.32 (1H, m, H6 or H9); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 210.9 (C, C1), 161.7 (CH, C3), 133.7 (CH, C2), 129.1 (CH, C7 or C8), 128.3 (CH, C7 or C8), 80.0 (CH, C4), 58.1 (C, C5), 43.0 (CH, C6 or C9), 37.4 (CH, C6 or C9); HRCIMS *m/z* = 150.0681 $[M]^+$ 0.2 ppm (150.0681 calcd $C_9H_{10}O_2$).
3.46: R_f 0.31 (1:1 petroleum ether:ethyl acetate, visualization UV/PMA); IR (thin film) ν_{max} 3440, 1728, 1334, 1164 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.71 (1H, m, H7 or H8), 5.56 (1H, m, H7 or H8), 4.16 (1H, t, 4.2 Hz, H4), 2.62 (1H, m, H6 or H9), 2.54-2.44 (3H, m, H6/9/2), 2.33 (1H, ddd, 19.2 Hz, 9.3 Hz, 4.5 Hz, H2), 2.27 (1H, m, H6 or H9), 2.16 (1H, m, H3), 1.99 (1H, ddd, 13.6 Hz, 9.4 Hz, 4.2 Hz, H3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 219.4 (C1), 129.6 (C7 or C8), 127.0 (C7 or C8), 77.5 (C4), 60.2 (C5), 41.2 (C6 or C9), 35.1 (C6 or C9), 33.3 (C2), 28.2 (C3); HRCIMS *m/z* = 153.0912 $[M+H]^+$ 2.5 ppm (153.0916 calcd for $C_9H_{13}O_2$).

O-^{t}Butyldimethylsilyl-4-hydroxy-*spiro*-nona-2,7-diene-1-one, 3.47

4-Hydroxy-*spiro*-nona-2,7-diene-1-one (**3.45**, 50 mg, 0.33 mmol) was dissolved in anhydrous DMF (400 μL), ^{t}BDMSCl (75 mg, 0.50 mmol, 1.5 eq) and imidazole (57 mg, 0.83 mmol, 2.5 eq) were added and the reaction stirred at room temperature for 16 hours. H_2O (2 mL) was added and the mixture was extracted with diethyl ether (3 x 2 mL). The combined organic phases were dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. The crude product was purified by chromatography on silica using a stepwise gradient of petroleum ether to 4% diethyl ether in petroleum ether, to give the product (**3.47**, 4 mg, 0.015 mmol, 5%) as a very volatile, colourless oil.
R_f 0.52 (4:1 petroleum ether:ethyl acetate, visualization UV/PMA); IR (thin film) ν_{max}1718, 1254, 1128, 872, 837 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.35 (1H, dd, 5.9 Hz, 2.2 Hz, H3), 6.22 (1H, dd, 5.9 Hz, 1.2 Hz, H2), 5.63 (2H, br s, H7/8), 4.59 (1H, br s, H4), 2.86 (1H, m, H6 or H9), 2.80 (1H, m, H6 or H9), 2.35 (1H, m, H6 or H9), 2.28 (1H, m,

H6 or H9), 0.90 (9H, s, H13), 0.12 (3H, s, H10), 0.07 (3H, s, H11); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 210.7 (C1), 162.2 (C3), 133.1 (C2), 129.3 (C7 or C8), 128.0 (C7 or C8), 80.7 (C4), 58.3 (C5), 42.9 (C6 or C9), 38.1 (C6 or C9), 25.7 (C13), -4.8 (C10), -4.9 (C11), C12 not observed; HRESIMS *m/z* = 265.1634 $[M+H]^+$ 3.7 ppm (265.1624 calcd for $C_{15}H_{25}SiO_2$).

_O_-Acetyl-4-hydroxy-*spiro*-nona-2,7-diene-1-one, 3.48

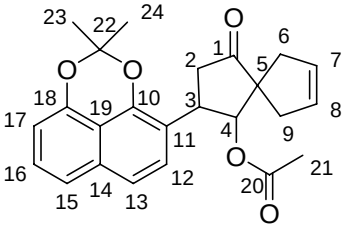
4-Hydroxy-*spiro*-nona-2,7-diene-1-one (**3.45**, 50 mg, 0.33 mmol) was dissolved in anhydrous pyridine (1 mL) and acetic anhydride (200 μL, 2.12 mmol, 6.4 eq) was added. The reaction was stirred at room temperature for 16 hours. Diethyl ether (6 mL) was added, the organic phase was washed with citric $acid_{(aq)}$ (5% w/v, 4 x 5 mL), dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. This gave the product (**3.48**, 59 mg, 0.31 mmol, 94%) as a colourless oil.

IR (thin film) ν_{max}1736, 1717, 1373, 1232, 1028 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.48 (1H, dd, 5.9 Hz, 2.3 Hz, H3), 6.37 (1H, dd, 5.9 Hz, 1.1 Hz, H2), 5.70 (1H, br s, H4), 5.64 (2H, m, H7/8), 2.77 (1H, m, H6 or H9), 2.60 (1H, br d, 17.2 Hz, H6 or H9), 2.52-2.47 (2H, m, H6 or H9), 2.11 (3H, s, H11); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 209.1 (C, C1), 170.1 (C, C10), 157.7 (CH, C3), 135.3 (CH, C2), 128.4 (CH, C7 or C8), 128.3 (CH, C7 or C8), 80.3 (CH, C4), 56.5 (C, C5), 43.2 (CH_2, C6 or C9), 38.0 (CH_2, C6 or C9), 20.8 (CH_3, C11); HRCIMS *m/z* = 193.0862 $[M+H]^+$ 1.4 ppm (193.0865 calcd for $C_{11}H_{13}O_3$).

1,8-Dihydroxy-3-[*O*-acetyl-4-hydroxy-*spiro*-nona-7-ene-1-one]-naphthalene-acetonide, 3.49

O-Acetyl-4-hydroxy-*spiro*-nona-2,7-diene-1-one (**3.48**, 20 mg, 0.10 mmol), 1,8-dihydroxynaphthalene acetonide (**3.39**, 30 mg, 0.15 mmol, 1.5 eq) and DHN (1 mg, 0.006 mmol, 6 mol%) were dissolved in anhydrous, degassed chloroform (2 mL) and triflic acid (3 μL, 0.034 mmol, 34 mol%) was added. The reaction was stirred at 45 ºC for 16 hours, then cooled to room temperature. $NaHCO_{3(aq)}$ (1 M, 3 mL) was added to the mixture and the phases separated. The aqueous phase was further extracted with diethyl ether (3 x 3 mL) and the combined organic phases were dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. The crude product was purified using chromatography on Florisil

using a stepwise gradient of petroleum ether to 10% diethyl ether in petroleum ether to give the product (**3.49**, ~0.5 mg, ~0.001 mmol, ~1%) as an amorphous solid.



R_f 0.41 (4:1 petroleum ether:ethyl acetate, visualization UV/PMA); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.43 (1H, d, 8.5 Hz, H15), 7.42-7.36 (2H, m, H16/H12 or H13), 7.32 (1H, d, 8.5 Hz, H12 or H13), 6.86 (1H, dd, 7.2 Hz, 1.2 Hz, H17), 5.80 (1H, d, 9.8 Hz, H4) 5.65 (1H, m, H7 or H8), 5.55 (1H, m, H7 or H8), 3.79 (1H, dd, 10.9 Hz, 9.5 Hz, H3), 2.99 (1H, dd, 19.2 Hz, 9.5 Hz, H2), 2.92 (1H, m, H6 or H9), 2.64 (1H, dd, 19.2 Hz, 10.9 Hz, H2), 2.55 (1H, m, H6 or H9), 2.40 (1H, m, H6 or H9), 1.92 (3H, s, H21), 1.68 (3H, s, H23 or H24), 1.67 (3H, s, H23 or H24); ^{13}C NMR ($CDCl_3$, from HSQC data at 500 MHz) δ 129.7 (CH, C7 or C8), 126.8 (CH, C12 or C16), 126.7 (CH, C7 or C8), 126.6 (CH, C12 or C16), 120.4 (CH, C13/15), 109.5 (CH, C17), 81.1 (CH, C4) 42.5 (CH_2, C2) 41.3 (CH_2, C6 or C9), 39.0 (CH, C3) 38.1 (CH_2, C6 or C9), 25.6 (CH_3, C23/24), 21.1 (CH_3, C21).

Section 3.2.4

_o_-Iodoxybenzoic acid, 3.50 [16]

o-Iodobenzene (10 g, 40.3 mmol) was added to a solution of oxone (74 g, 120.4 mmol, 3 eq) in deionised H_2O (400 mL) and stirred at 70 ºC for 90 minutes. The reaction was then cooled in an ice-bath and the precipitate formed was filtered, washed with H_2O (4 x 10 mL) and acetone (2 x 10 mL), and residual solvents were removed *in vacuo*. This gave the product (**3.50**, 8.06 g, 28.8 mmol, 71%) as a white solid.

mp 224-228 ºC (decomposition observed at this temperature; lit. 233 ºC);[16] IR (diffuse reflectance) ν_{max} 3300-3000, 1645, 1332, 1300 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.24 (1H, d, 7.9 Hz), 8.13 (1H, d, 7.4 Hz), 8.10 (1H, t, 7.9 Hz), 7.94 (1H, t, 7.4 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.7, 146.7, 133.6, 133.2, 131.6, 130.3, 125.2.

spiro-Nona-2,7-diene-1,4-dione-1,1-[1,8-dihydroxynaphthalene]-acetal (or 4-keto-6,9-deoxy-_spiro_-mamakone A), 3.34

spiro-Nona-7-ene-1,4-dione-1,1-[1,8-dihydroxynaphthalene]-acetal (**3.35**, 95 mg, 0.33 mmol), IBX (**3.50**, 380 mg, 1.36 mmol, 4.1 eq) and *N*-methoxypyridine-*N*-oxide hydrate (170 mg, 1.36 mmol, 4.1 eq) were dissolved in DMSO (1.4 mL) over around 15 minutes to form a clear reddish solution. The reaction was then stirred at 70°C for 20 hours during which time a white precipitate formed. $NaHCO_{3\,(aq)}$ was slowly added (1M, approximately 2 mL, effervescence observed and white IBX derivatives precipitate out of solution) and the resulting mixture was extracted with diethyl ether (4 x 3 mL). The combined organic phases were dried over $MgSO_4$ and filtered. The crude product was then purified by chromatography on deacidified silica using a stepwise gradient of petroleum ether and diethyl ether (0 to 2% diethyl ether). The product was eluted first with 0.5 % ether and any remaining starting material eluted after with 1%. The product was obtained as a white solid (**3.34**, isolated yield 40 mg, 42%).

mp 142-144 ºC (recrystallised from diethyl ether); R_f 0.56 (4:1 petroleum ether:ethyl acetate, visualization UV/PMA); IR (diffuse reflectance) ν_{max} 1725, 1611, 1411, 1278, 1262, 1250 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.51 (2H, dd, 8.4 Hz, 0.7 Hz, H13/15), 7.43 (2H, dd, 8.4 Hz, 7.5 Hz, H12/16), 7.38 (1H, d, 6.1 Hz, H2 or H3), 6.93 (2H, dd, 7.5 Hz, 0.7 Hz, H11/17), 6.39 (1H, d, 6.1 Hz, H2 or H3), 5.65 (2H, br s, H7/8), 3.22 (2H, br d, 16 Hz, H6/9), 2.64 (2H, br d, 16 Hz, H6/9); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 206.6 (C, C4), 153.3 (CH, C2), 147.6 (C, C10/18), 135.2 (CH, C3), 134.2 (C, C14), 128.0 (CH, C7/8), 127.5 (CH, C12/C16), 120.9 (CH, C13/C15), 113.7 (C, C19), 109.3 (CH, C11/C17), 105.8 (C, C1), 61.2 (C, C5), 38.0 (CH_2, C6/C9); HRESIMS *m/z* = 291.1030 ± 2.0 ppm $[M+H]^+$ (291.1021 calcd for $C_{19}H_{15}O_3$).

Dehydrogenation with concomitant olefin rearrangement to give _spiro_-nona-2,6-diene-1,4-dione-1,1-[1,8-dihydroxynaphthalene]-acetal, 3.53

spiro-Nona-7-ene-1,4-dione-1,1-[1,8-dihydroxynaphthalene]-acetal (**3.35**, 72 mg, 0.25 mmol), IBX (**3.50**, 240 mg, 0.86 mmol, 3.4 eq) and *N*-methoxypyridine-*N*-oxide hydrate (106 mg, 0.86 mmol, 3.4 eq) were dissolved in DMSO (1.4 mL) over around 15 minutes to form a clear reddish solution. The reaction was then stirred at 55°C for 16 hours

during which time a white precipitate formed. $NaHCO_{3\,(aq)}$ was slowly added (1M, approximately 2 mL, effervescence observed and white IBX derivatives precipitate out of solution) and the resulting mixture was extracted with diethyl ether (4 x 3 mL). The combined organic phases were dried over $MgSO_4$ and filtered. The crude product was then purified by chromatography on deacidified silica using a stepwise gradient of petroleum ether and diethyl ether (0 to 2% diethyl ether) to give a mixture of **3.34** and **3.53** (14mg, 0.05 mmol). A portion of the mixture (~ 4 mg) was separated by chromatography on an analytical C_{18} HPLC column using the following elution system; solvents: A: H_2O, B: MeCN; isocratic: 0 min 55% B, linear gradient: 2 min 55% B, 23 min 65% B; 25 ºC; 1 mL min^{-1}. **3.53**: R_t 19.42 min, 1.0 mg; **3.34**: R_t 20.76 min, 2.5 mg. **3.53**: IR (diffuse reflectance) ν_{max} 1725, 1612, 1412, 1276 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.50 (2H, m, H13/15), 7.47 (1H, d, 6.1 Hz, H2), 7.44-7.40 (2H, m, H12/16), 6.93 (1H, dd, 7.5 Hz, 0.8 Hz, H11 or H17), 6.90 (1H, dd, 7.6 Hz, 0.7 Hz, H11 or H17), 6.45 (1H, d, 6.2 Hz, H3), 6.01 (1H, dt, 5.8 Hz, 2.3 Hz, H6 or H7), 5.63 (1H, dt, 5.7 Hz, 2.1 Hz, H6 or H7), 2.58-2.48 (2H, m), 2.24-2.13 (2H, m); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 206.7 (C, C4), 154.0 (CH, C2), 147.5 (C, C10/18), 136.5 (CH, C6 or C7), 135.6 (CH, C3), 134.2 (C, C14), 128.8 (CH, C6 or C7), 127.5 (CH, C12 or C16), 127.4 (CH, C12 or C16), 120.8 (CH, C13 or C15), 120.75 (CH, C13 or C15), 113.6 (C, C19), 109.4 (CH, C11 or C17), 109.1 (CH, C11 or C17), 106.2 (C, C1), 69.6 (C, C5), 32.3 (CH_2, C8 or C9), 29.1(CH_2, C8 or C9); HRESIMS *m/z* = 291.1022 ± 0.3 ppm $[M+H]^+$ (291.1021 calcd for $C_{19}H_{15}O_3$).

4-Hydroxy-*spiro*-nona-7-ene-1-one-1,1-[1,8-dihydroxynaphthalene]-acetal (or 6,8-deoxy-2,3-dihydro-*spiro*-mamakone A), 3.55

spiro-Nona-7-ene-1,4-dione-1,1-[1,8-dihydroxynaphthalene]-acetal (**3.35**, 10 mg, 0.034 mmol), was dissolved in MeOH (1 mL) and $NaBH_4$ (7.5 mg, 0.20 mmol, 5.9 eq) was added. The reaction was stirred at room temperature for 16 hours, then $NaHCO_{3\,(aq)}$ (1M, 2 mL) was added and the reaction extracted with ethyl acetate (3 x 2 mL). After drying over $MgSO_4$, filtration and evaporation of solvent, the crude product was purified on silica (deacidified) using a stepwise gradient of petroleum ether to 10% diethyl ether in petroleum ether, to give the product as a colourless oil (**3.55**, 4.8 mg, 0.016 mmol, 47%).

R_f 0.26 (4:1 petroleum ether:ethyl acetate, visualization UV/PMA); IR (thin film) ν_{max} 3406, 2926, 1609, 1411, 1381, 1275 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.47 (1H, dd, 8.3 Hz, 0.9 Hz, H13 or H15), 7.46 (1H, dd, 8.4 Hz, 0.9 Hz, H13 or H15), 7.40 (1H, dd, 8.3 Hz, 7.5 Hz, H12 or H16), 7.395 (1H, dd, 8.4 Hz, 7.4 Hz, H12 or H16), 6.89 (1H, dd, 7.5 Hz, 1.0 Hz, H11 or H17), 6.88 (1H, dd, 7.4 Hz, 0.9 Hz, H11 or H17), 5.77 (1H, m, H7 or H8), 5.69 (1H, m, H7 or H8), 4.08 (1H, m, H4), 3.05 (1H, m, H6 or H9), 2.86 (1H, m, H6 or H9), 2.76 (1H, m, H6 or H9), 2.48 (1H, d, 9.34 Hz, OH), 2.31 (1H, m, H3), 2.15 (1H, m, H6 or H9), 2.06 (1H, m, H2), 1.80 (2H, m, H2/3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 148.4 (C, C10 or C18), 147.6 (C, C10 or C18), 134.4 (C, C14), 129.9 (CH, C7 or C8), 127.8 (CH, C7 or C8), 127.3 (CH, C12 or C16), 127.2 (CH, C12 or C16), 120.6 (CH, C13 or C15), 120.4 (CH, C13 or C15), 114.0 (C, C19), 112.0 (C, C1), 109.3 (CH, C11 or C17), 109.2 (CH, C11 or C17), 77.8 (CH, C4), 58.9 (C, C5), 39.2 (CH_2, C6 or C9), 32.7 (CH_2, C6 or C9), 30.7 (CH_2, C2), 29.7 (CH_2, C3); HRESIMS *m/z* 295.1339 $[M+H]^+$ 1.7 ppm (295.1334 calcd for $C_{19}H_{19}O_3$).

4-Hydroxy-*spiro*-nona-2,7-diene-1-one-1,1-[1,8-dihydroxynaphthalene]-acetal (or 6,9-deoxy-*spiro*-mamakone A), 3.54

spiro-Nona-2,7-diene-1,4-dione-1,1-[1,8-dihydroxynaphthalene]-acetal (**3.34**, 10 mg, 0.034 mmol) and $CeCl_3$ (anhydrous, 9 mg, 0.036 mmol, 1.1 eq) were dissolved in MeOH (500 μL) and cooled to 0 ºC. $NaBH_4$ (1.3 mg, 0.034 mmol, 1 eq) was added and the reaction stirred at 0 ºC for 1 hour. $NaHCO_{3\ (aq)}$ (1 M, 1 mL) was added and the reaction extracted with diethyl ether (4 x 1 mL). The combined organic phases were dried over $MgSO_4$, filtered and the solvent was removed *in vacuo*. The crude product was purified by chromatography on deacidified silica using a stepwise gradient of petroleum ether to 12% diethyl ether in petroleum ether, to give the product as a colourless oil (**3.54**, 10 mg, 0.034 mmol, ~100%).

R_f 0.31 (4:1 petroleum ether:ethyl acetate, visualization UV/PMA); IR (thin film) ν_{max} 1607, 1413, 1382, 1277, 1100 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.47 (1H, dd, 5.1 Hz, 0.8 Hz, H13 or H15), 7.45 (1H, dd, 5.3 Hz, 0.7 Hz, H13 or H15), 7.40 (1H, t, 7.9 Hz, H12 or H16), 7.38 (1H, t, 7.9 Hz, H12 or H16), 6.91 (1H, dd, 7.5 Hz, 0.8 Hz, H11 or H17), 6.83 (1H, dd, 7.5 Hz, 0.8 Hz, H11 or H17), 6.35 (1H, dd, 6.0 Hz, 2.7 Hz, H3), 6.02 (1H, d,

6.0 Hz, H2), 5.76 (1H, m, H7 or H8), 5.69 (1H, m, H7 or H8), 4.39 (1H, d, 2.7 Hz, H4), 3.19 (1H, ddd, 16.9 Hz, 2.6 Hz, 2.4 Hz, H6 or H9), 2.85 (2H, m, H6/H9), 2.09 (1H, 16.9 Hz, 1.9 Hz, 1.6 Hz, H6 or H9); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 148.6 (C, C10 or C18), 148.3 (C, C10 or C18), 140.3 (CH, C3), 134.3 (C, C14), 132.9 (CH, C2), 129.5 (CH, C7 or C8), 128.2 (CH, C7 or C8), 127.3 (CH, C12/C16), 120.5 (CH, C13 or C15), 120.4 (CH, C13 or C15), 114.1 (C, C19), 111.0 (C, C1), 109.2 (CH, C11 or 17), 108.8 (CH, C11 or C17), 81.1 (CH, C4), 59.9 (C, C5), 40.3 (CH_2, C6/C9); HRESIMS *m/z* = 293.1186 $[M+H]^+$ 3 ppm (293.1178 calcd for $C_{19}H_{17}O_3$).

_O_-Acetyl-4-hydroxy-_spiro_-nona-2,7-diene-1-one-1,1-[1,8-dihydroxynaphthalene]-acetal (or _O_-Acetyl-6,9-deoxy-_spiro_-mamakone A), 3.56

4-Hydroxy-*spiro*-nona-2,7-diene-1-one-1,1-[1,8-dihydroxynaphthalene]-acetal (**3.54**, 5 mg, 0.017 mmol) was dissolved in pyridine (500 μL) and acetic anhydride was added (200 μL). The reaction was stirred at room temperature for 20 hours, then diethyl ether was added (2 mL) and the mixture extracted with $NaHCO_{3\ (aq)}$ (1M, 3 x 2 mL). After drying over $MgSO_4$, filtration and evaporation of solvent, the crude product was purified on silica (deacidified) using a stepwise gradient of 0 to 12% diethyl ether in petroleum ether, to give the product (**3.56**, 5.5 mg, ~ 100%) as a colourless oil.

R_f 0.69 (4:1 petroleum ether:ethyl acetate, visualization UV/PMA); IR (thin film) ν_{max} 1738, 1607, 1412, 1381, 1279, 1219 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.46 (1H, dd, 8.4 Hz, 0.9 Hz, H13 or H15), 7.45 (1H, dd, 8.4 Hz, 0.9 Hz, H13 or H15), 7.40 (1H, dd, 7.5 Hz, 8.4 Hz, H12 or H16), 7.39 (1H, dd, 8.3 Hz, 7.5 Hz, H12 or H16), 6.91 (1H, dd, 7.5 Hz, 0.9 Hz, H11 or H17), 6.86 (1H, dd, 7.4 Hz, 0.9 Hz, H11 or H17), 6.17 (1H, dd, 6.1 Hz, 2.2 Hz, H3), 6.05 (1H, dd, 6.0 Hz, 1.2 Hz, H2), 5.74 (1H, dd, 2.1 Hz, 1.2 Hz, H4), 5.68 (2H, m, H7/8), 3.18 (1H, m, H6 or H9), 2.97 (1H, m, H6 or H9), 2.60 (1H, m, H6 or H9), 2.45 (1H, m, H6 or H9), 2.11 (3H, s, H21); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.8 (C, C20), 148.8 (C, C10 or C18), 148.5 (C, C10 or C18), 136.8 (CH, C3), 134.3 (C, C14), 133.2 (CH, C2), 128.7 (CH, C7 or C8),128.4 (CH, C7 or C8), 127.32 (CH, C12 or C16), 127.28 (CH, C12 or C18), 120.4 (CH, C13 or C15), 120.3 (CH, C13 or C15), 114.1 (C, C19), 109.9 (C, C1), 108.9 (CH, C11/C17), 82.1 (CH, C4), 60.5 (C, C5), 38.9 (CH_2, C6

or C9), 34.3 (CH_2, C6 or C9), 21.1 (CH_3, C21); HRESIMS *m/z* = 335.1293 $[M+H]^+$ 3 ppm (335.1283 calcd for $C_{21}H_{19}O_4$).

Dimethylpyrazole (DMP)[17]

Acetylacetone (8 mL, 78 mmol) was added slowly to hydrazine hydrate (20 mL, 640 mmol, 8.2 eq) with vigorous stirring and a white precipitate was formed. The reaction was stirred for 15 minutes, then the precipitate was filtered, washed with hexane (3 x 20 mL) and all solvents removed *in vacuo* to give the product (3.36 g, 28.6 mmol, 37%) as a white solid.

mp 105-108 ºC (lit – 107-108 ºC);[18] ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.25 (1H, br s, NH), 5.85 (1H, s), 2.27 (6H, s).[19]

Pearlman's catalyst ($Pd(OH)_2/C$)[20]

$PdCl_2$ (300 mg, 1.7 mmol) and activated charcoal (720 mg) in deionised H_2O (6 mL) were heated to 80 ºC and $LiOH.H_2O$ (150 mg, 3.6 mmol, 2.1 eq) was added in one portion. Heating was stopped and the reaction was stirred at room temperature for 16 hours. The product was filtered and washed with 0.5 % acetic $acid_{(aq)}$ (w/v) and residual solvent removed *in vacuo* at 70 ºC for 4 hours.

***spiro*-Nona-6-ene-1,4,8-trione-1,1-[1,8-dihydroxynaphthalene]-acetal, 3.78 (erroneously assigned as 3.57)**

spiro-Nona-7-ene-1,4-dione-1,1-[1,8-dihydroxynaphthalene]-acetal (**3.35**, 12 mg, 0.041 mmol), dirhodium(II)tetrakis(caprolactam) (0.3 mg, 0.45 μmol, 1 mol%) and $NaHCO_3$ (1.5 mg, 0.018 mmol, 0.4 eq) were suspended in anhydrous DCM (1 mL). ^{t}Butyl hydroperoxide 5M in decanes (40 μL, 0.20 mmol, 5 eq) was added. The reaction was stirred at room temperature for 16 hours, then filtered through a silica (deacidified) pad using DCM. The crude product was further purified on silica (deacidified) using a stepwise gradient of petroleum ether to 24% diethyl ether in petroleum ether, to give product (**3.78**, 5.5 mg, 0.018 mmol, 44%) as a colourless oil and recovered starting material (**3.35**, 3 mg, 0.010 mmol, 24%).

R_f 0.23 (4:1 petroleum ether:ethyl acetate, visualization UV/PMA); IR (thin film) ν_{max} 1750, 1718, 1609, 1270 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.55 (1H, d, 5.7 Hz, H8), 7.523 (1H, dd, 8.3 Hz, 0.7 Hz, H13 or H15), 7.518 (1H, dd, 8.4 Hz, 0.8 Hz, H13 or H15), 7.44 (1H, dd, 8.3 Hz, 7.6 Hz, H12 or H16), 7.43 (1H, dd, 8.3 Hz, 7.6 Hz, H12 or H16), 6.93 (1H, dd, 6.6 Hz, 0.8 Hz, H11 or H17), 6.92 (1H, dd, 6.6 Hz, 0.8 Hz, H11 or H17), 6.36 (1H, d, 5.7 Hz, H7), 2.94 (1H, d, 18.3 Hz, H9), 2.69 (2H, m, H2 or H3), 2.50 (1H, d, 18.3 Hz, H9), 2.46 (1H, ddd, 14.0 Hz, 9.4 Hz, 6.0 Hz, H2 or H3), 2.33 (1H, ddd, 14.0 Hz, 9.4 Hz, 7.4 Hz, H2 or H3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 210.4 (C, C4), 205.9 (C, C6), 157.6 (CH, C8), 146.8 (C, C10 or C18), 146.6 (C, C10 or C18), 136.6 (CH, C7), 134.2 (C, C14), 127.5 (CH, C12 or C16), 127.4 (CH, C12 or C16), 121.2 (CH, C13/C15), 113.5 (C, C19), 109.6 (CH, C11 or C17), 109.4 (CH, C11 or C17),107.1 (C, C1), 66.8 (C, C5), 38.3 (CH_2, C9), 35.1 (CH_2, C2 or C3), 29.1 (CH_2, C2 or C3); HRESIMS *m/z* = 307.0979 $[M+H]^+$ 3.0 ppm (307.0970 calcd for $C_{19}H_{15}O_4$).

spiro-Nona-2,6-diene-1,4,8-trione-1,1-[1,8-dihydroxynaphthalene]-acetal, 3.77 (erroneously assigned as 3.58)

spiro-Nona-2,7-diene-1,4-dione-1,1-[1,8-dihydroxynaphthalene]-acetal (**3.34**, 10 mg, 0.034 mmol), dirhodium(II)tetrakis(caprolactam) (0.2 mg, 0.3 μmol,1 mol%) and $NaHCO_3$ (1.4 mg, 0.017 mmol, 0.5 eq) were suspended in anhydrous DCM (1 mL). ^{t}Butyl hydroperoxide 5M in decanes (35 μL, 0.17 mmol, 5 eq) was added and an immediate colour change from light purple to deep pink was observed. The reaction was stirred at room temperature for 20 hours, then filtered through a silica (deacidified with TEA) pad using DCM. The crude product was further purified on silica (deacidified) using a stepwise gradient of petroleum ether to 16% diethyl ether in petroleum ether, to give the product (**3.77**, 3.4 mg, 34%) as a colourless oil.

R_f 0.20 (4:1 petroleum ether:ethyl acetate, visualization UV/PMA); IR (think film) ν_{max} 1732, 1725, 1609, 1581, 1411, 1378, 1270 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.59 (1H, d, 6.0 Hz, H2), 7.56 (1H, d, 5.6 Hz, H8), 7.55 (2H, m, H13/15), 7.454 (1H, dd, 8.4 Hz, 7.6 Hz, H12 or H16), 7.448 (1H, dd, 8.4 Hz, 7.6 Hz, H12 or H16), 6.97 (1H, dd, 7.5 Hz, 0.8 Hz, H11 or H17), 6.95 (1H, dd, 7.5 Hz, 0.8 Hz, H11 or H17), 6.52 (1H, d, 6.0 Hz, H3), 6.33 (1 H, d, 5.6 Hz, H7), 3.09 (1H, d, 18.7 Hz, H9), 2.62 (1H, d, 18.7 Hz, H9); ^{13}C

NMR ($CDCl_3$, 75 MHz) δ 206.5 (C, C6), 201.5 (C, C4), 159.1 (CH, C8), 154.3 (CH, C2), 146.6 (C, C10/18), 135.4 (CH, C7), 135.1 (CH, C3), 134.2 (C, C14), 127.6 (CH, C12 or C16), 127.5 (CH, C12 or C16), 121.52 (CH, C13 or C15), 121.49 (CH, C13 or C15), 109.6 (CH, C11/17), 105.3 (C, C1), 65.0 (C, C5), 39.0 (CH_2, C9), C19 not observed; HRESIMS *m/z* = 305.0821 $[M+H]^+$ 1 ppm (305.0814 calcd for $C_{19}H_{13}O_4$).

_O_-Acetyl-4-hydroxy-*spiro*-nona-2,6-diene-1,8-dione-1,1-[1,8-dihydroxynaphthalene]-acetal, 3.79 ([4*R*,5*R*] and [4*S*,5*S*]) and 3.80 ([4*R*,5*S*] and [4*S*,5*R*]). (erroneously assigned as 3.59 and 3.60)

O-Acetyl-4-hydroxy-*spiro*-nona-2,7-diene-1-one-1,1-[1,8-dihydroxynaphthalene]-acetal (**3.56**, 5 mg, 0.015 mmol), dirhodium(II)tetrakis(caprolactam) (0.1 mg, 0.15 μmol, 1 mol%) and $NaHCO_3$ (0.7 mg, 0.008 mmol, 0.5 eq) were suspended in anhydrous DCM (1 mL). ^{t}Butyl hydroperoxide 5M in decanes (20 μL, 0.08 mmol, 5 eq) was added and an immediate colour change from light purple to deep pink was observed. The reaction was stirred at room temperature for 16 hours, then filtered through a silica (deacidified with TEA) pad using DCM. The crude product was further purified on silica (deacidified) using a stepwise gradient of 8 to 16% diethyl ether in petroleum ether, to give **3.79** (2.1 mg, 0.0060 mmol, 40 %) and **3.80** (0.8 mg, 0.0023 mmol, 15%) as colourless oils.

3.79: R_f 0.18 (4:1 petroleum ether:ethyl acetate, visualization UV/PMA); IR (thin film) ν_{max} 1742, 1723, 1607, 1412, 1225 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.80 (1H, d, 5.7 Hz, H8), 7.49 (1H, dd, 8.4 Hz, 0.9 Hz, H13 or H15), 7.48 (1H, dd, 8.4 Hz, 0.9 Hz, H13 or H15) 7.41 (2H, t, 8.2 Hz, H12/16), 6.93 (1H, dd, 7.5 Hz, 0.9 Hz, H11 or H17), 6.89 (1H, dd, 7.5 Hz, 0.9 Hz, H11 or H17), 6.24 (1H, dd, 6.1 Hz, 1.7 Hz, H3), 6.21 (1H, d, 5.7 Hz, H7), 6.11 (1H, dd, 6.0 Hz, 1.9 Hz, H2), 6.05 (1H, t, 1.8 Hz, H4), 3.06 (1H, d, 18.8 Hz, H9), 2.63 (1H, d, 18.8 Hz, H9), 2.11 (3H, s, H21); ^{13}C NMR ($CDCl_3$, from 2D spectra at 500 MHz) δ 207.6 (C, C6), 170.5 (C, C20), 162.2 (CH, C8), 148.3 (C, C10 or C18), 147.6 (C, C10 or C18), 137.1 (CH, C3), 136.0 (CH, C7), 134.4 (C, C14), 132.5 (CH, C2), 127.5 (CH, C12/16), 121.1 (CH, C13/15), 113.8 (C, C19), 109.5 (C/CH, C11/C17/C1), 78.0 (CH, C4), 63.5 (C, C5), 37.3 (CH2, C9), 20.9 (CH_3, C21); HRESIMS *m/z* = 349.1081 $[M+H]^+$ 1.4 ppm (349.1076 calcd for $C_{21}H_{17}O_5$).

3.80: R_f 0.16 (4:1 petroleum ether:ethyl acetate, visualization UV/PMA); IR (thin film) ν_{max} 1740, 1722, 1608, 1412, 1221 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.58 (1H, d, 5.8 Hz, H7), 7.50 (1H, dd, 8.3 Hz, 0.7 HzH13 or H15), 7.48 (1H, dd, 8.3 Hz, 0.8 Hz, H13 or H15), 7.41 (1H, dd, 8.3 Hz, 7.6 Hz, H12 or H16), 7.40 (1H, dd, 8.3 Hz, 7.5 Hz, H12 or H16), 6.89 (1H, dd, 7.6 Hz, 0.9 Hz, H11 or H17), 6.87 (1H, dd, 7.6 Hz, 0.8 Hz, H11 or H17), 6.28 (1H, d, 5.8 Hz, H8), 6.24 (1H, dd, 6.1 Hz, 1.8 Hz, H2 or H3), 6.13 (1H, dd, 6.1 Hz, 1.6 Hz, H2 or H3), 5.95 (1H, t, 1.6 Hz, H4), 3.2 (1H, d, 19.0 Hz, H6), 2.74 (1H, d, 19.0 Hz, H6), 2.07 (3H, s, H21); ^{13}C NMR ($CDCl_3$, from 2D spectra at 500 MHz) δ 207.8 (C, C9), 170.4 (C, C20), 161.8 (CH, C7), 147.8 (C, C10/18), 136.6 (CH, C2 or C3), 135.1 (CH, C8), 134.5 (C, C14), 132.3 (CH, C2 or C3), 127.6 (CH, C12/16), 121.0 (CH, C13/15), 113.9 (C, C19), 109.5 (CH, C11/17), 109.3 (C, C1), 81.6 (CH, C4), 63.4 (C, C5), 40.3 (CH_2, C6), 21.0 (CH_3, C21); HRESIMS *m/z* = 349.1082 $[M+H]^+$ 1.7 ppm (349.1076 calcd for $C_{21}H_{17}O_5$).

Section 3.2.5

7,8-Dimethyl-*spiro*-nona-7-ene-1,4-dione-1,1-[1,8-dihydroxynaphthalene]-acetal, 3.63

2,2-Diallylcyclopentane-1,3-dione (**3.26**, 100 mg, 0.56 mmol), 1,8-dihydroxynaphthalene acetonide (**3.39,** 170 mg, 0.84 mmol, 1.5 eq) and DHN (5 mg, 0.03 mmol, 5 mol%) were dissolved in anhydrous, degassed chloroform (1 mL) and triflic acid (20 μL, 0.22 mmol, 0.4 eq) was added. The reaction was stirred at 40 ºC for 40 hours, then cooled to room temperature. $NaHCO_{3(aq)}$ (1 M, 3 mL) was added to the mixture and the phases separated. The aqueous phase was further extracted with diethyl ether (3 x 3 mL) and the combined organic phases were dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. The crude product was purified using chromatography on Florisil using a stepwise gradient of 0 to 12% diethyl ether in petroleum ether to give the product (**3.61**, 3.5 mg, 0.011 mmol, 2%) as a white amorphous solid.

R_f 0.54 (4:1 petroleum ether:ethyl acetate, visualization UV/PMA); IR (diffuse reflectance) ν_{max} 1750, 1609, 1411, 1380, 1271, 1146 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.48 (2H, dd, 8.3 Hz, 0.7 Hz, H13/15), 7.41 (2H, dd, 8.3 Hz, 7.5 Hz, H12/16), 6.90 (2H,

dd, 7.5 Hz, 0.7 Hz, H11/17), 2.97 (2H, br d, 15.5 Hz, H6/9), 2.51 (2H, t, 7.9 Hz, H2 or H3), 2.48 (2H, br d, 15.5 Hz, H6/9), 2.15 (2H, t, 7.9 Hz, H2 or H3), 1.66 (6H, br s, H6Me/H7Me); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 214.5 (C, C4), 147.6 (C, C10/18), 134.3 (C, C14), 128.4 (C, C7/8), 127.3 (CH, C12/16), 120.6 (CH, C13/15), 113.9 (C, C19), 109.3 (CH, C11/17), 108.2 (C, C1), 60.3 (C, C5), 41.4 (CH_2, C6/9), 33.7 (CH_2, C2 or C3), 28.0 (CH_2, C2 or C3), 13.6 (CH_3, C6Me/C7Me); HRESIMS *m/z* = 321.1489 $[M+H]^+$ 0.6 ppm (321.1491 calcd for $C_{21}H_{21}O_3$).

1,3-Dihydroxy-2,2-diallylcyclopentane, 3.67

2,2-Diallylcyclopentane-1,3-dione (**3.26**, 45 mg, 0.25 mmol) was dissolved in anhydrous MeOH (2 mL) and the mixture was cooled in an ice bath. $NaBH_4$ (30 mg, 0.94 mmol, 3.8 eq) was added and the reaction allowed to warm to room temperature with stirring. After 2 hours deionised H_2O (3 mL) was added and the mixture extracted with diethyl ether (4 x 3 mL). The combined organic phases were dried over $MgSO_4$, filtered and reduced *in vacuo*. The crude residue was purified using chromatography on silica using a step-wise gradient of 5 to 40% diethyl ether in petroleum ether. *cis*-1,3-Dihydroxy-2,2-diallylcyclopentane (*cis*-**3.67**, 14.3 mg, 0.078 mmol, 31%) and *trans*-1,3-dihydroxy-2,2-diallylcyclopentane (*trans*-**3.67**, 23.7 mg, 0.13 mmol, 52%) were obtained as a colourless oils.

cis-**3.67**: R_f 0.35 (2:1 petroleum ether:ethyl acetate, visualization UV/PMA); IR (thin film) ν_{max} 3500-3200, 2958, 1456, 1043 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.03-5.89 (1H, m, H7 or H10), 5.84-5.73 (1H, m, H7 or H10), 5.21-5.00 (4H, m, H8 and H11), 3.88 (2H, br s, H1 and H4), 2.48 (2H, br t, 7.2Hz, H6 and H9), 2.17-2.07 (4H, m, H2 and H3), 1.91-1.83 (2H, m, H6 and H9); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 136.0 (CH, C7/10), 135.8 (CH, C7/C10), 117.5 (CH_2, C8/C11), 117.0 (CH_2, C8/C11), 79.0 (CH, C1/C4), 78.7 (CH, C1/C4), 53.1 (C, C5), 53.0 (C, C5), 37.2 (CH_2, C6/C9), 35.2 (CH_2, C6/C9), 31.8 (CH_2, C2/C3), 31.6 (CH_2, C2/C3).

trans-**3.67**: R_f 0.19 (2:1 petroleum ether:ethyl acetate, visualization UV/PMA); IR (thin film) ν_{max} 3500-3200, 2958, 1441, 1024 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.00 (2H, m, H7 and H10), 5.14 (4H, m, H8 and H11), 4.12 (2H, m, H1 and H4), 2.34 (2H, m, H6 and H9), 2.16 (4H, m, H6, H9, H2 and H3), 1.52 (2H, m, H2 and H3); ^{13}C NMR ($CDCl_3$, 75

MHz) δ 135.6 (CH, C7/C10), 117.7 (CH2, C8/C11), 78.9 (CH, C1/C4), 35.6 (CH_2, C6/C9), 29.7 (CH_2, C2/C3); HRCIMS *m/z* = 183.1384 $[M+H]^+$ 0.7 ppm (183.1385 calcd for $C_{11}H_{19}O_2$).

_O,O_-Diacetyl-_trans_-1,3-dihydroxy-2,2-diallylcyclopentane, *trans*-**3.68**

trans-1,3-Dihydroxy-2,2-diallylcyclopentane (*trans*-**3.67**, 23 mg, 0.13 mmol) was dissolved in anhydrous pyridine (500 μL) and acetic anhydride was added (200 μL, 2.1 mmol, 16 eq). The reaction was stirred at room temperature for 16 hours. Diethyl ether (4 mL) was added and the mixture was extracted with citric $acid_{(aq)}$ (5% w/v, 3 x 4 mL). The organic phase was dried over $MgSO_4$, filtered and reduced *in vacuo* to give the product (*trans*-**3.68**, 21 mg, 0.079 mmol, 61%) as a colourless oil.
IR (thin film) ν_{max} 1739, 1238, 1208 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.82 (2H, m, H7 and H10), 5.10-5.03 (4H, m, H8 and H11), 2.34 (2H, m, H6 and H9), 2.26 (2H, m, H2 and H3), 2.12 (2H, m, H6 and H9), 2.04 (6H, s, H13 and H15), 1.58 (2H, m, H2 and H3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.5 (C12/C14), 134.0 (C7/C10), 117.9 (C8/C11), 79.5 (C1/C4), 49.6 (C5), 36.0 (C6/C9), 27.7 (C2/C3), 21.2 (C13/C15); HRCIMS *m/z* = 267.1594 $[M+H]^+$ 0.8 ppm (267.1596 calcd for $C_{15}H_{23}O_4$).

_O,O_-Diacetyl-_trans_-1,3-dihydroxy-2,2-di(allylcarboxylic acid) cyclopentane, 3.70 and _O,O_-diacetyl-_trans_-1,3-dihydroxy-2-allyl-2-(allylcarboxylic acid) cyclopentane, 3.71

O,O-Diacetyl-*trans*-1,3-dihydroxy-2,2-diallylcyclopentane (18 mg, 0.068 mmol) was dissolved in DCM (1 mL) and dirhodium(II)tetrakis(caprolactam) (0.5 mg, 0.68 μmol, 1 mol%) and $NaHCO_3$ (3 mg, 0.034 mmol, 0.5 eq) were added. ^{t}Butyl hydroperoxide (5M in decanes, 0.5 mL, 2.5 mmol, 37 eq) was added and the reaction stirred at room temperature for 16 hours. The solvents were removed *in vacuo*, and the crude residue was purified chromatography on silica using a step-wise gradient of 0 to 40% diethyl ether in petroleum ether. Two products were isolated, the first of which, **3.70**, required further purification by chromatography on silica using 10 to 30% diethyl ether in petroleum ether. This gave the product (**3.70**, ~ 0.1 mg, 0.3 μmol, 0.4%) as a colourless oil. A second product, **3.71**, was not obtained in sufficient quantity to attempt further purification, however was tentatively assigned as **3.71**.

3.70; R_f 0.36 (2:1 petroleum ether:ethyl acetate, visualization PMA); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.01 (2H, d, 16.6 Hz, H6 and H9), 5.78 (2H, d, 16.5 Hz, H7 and H10), 5.19 (2H, dd, 6.5 Hz, 3.3 Hz, H1 or H4), 5.08 (2H, t, 6.5 Hz, H1 or H4), 2.21 (2H, m, H2/3), 2.01 (3H, s, H13 or H15), 2.00 (3H, s, H13 or H15), 1.62-1.53 (2H, m, H2/3); ^{13}C NMR ($CDCl_3$, from HSQC and HMBC data at 500 MHz) δ 170.8 (C, C12 or C14), 170.6 (C, C12 or C14), 164.5 (C, C8 and C11), 149.6 (CH, C6 and C9), 117.4 (CH, C 7 and C10), 79.2 (CH, C1 or C4), 76.7 (CH, C1 or C4), 27.8 (CH_2, C2 and C3), 27.5 (CH_2, C2 and C3), 21.4 (CH_3, C13/15), C5 not observed.
3.71: R_f 0.18 (2:1 petroleum ether:ethyl acetate, visualization PMA); HRESIMS *m/z* = 295.1178 ± 1.4 ppm $[M-H]^-$ (295.1182 calcd for $C_{15}H_{19}O_6$).

Section 3.2.6

_O_-Acetyl-4,8-dihydroxy-*spiro*-nona-2,6-diene-1-one-1,1-[1,8-dihydroxynaphthalene]-acetal, 3.81

O-Acetyl-4-hydroxy-*spiro*-nona-2,6-diene-1,8-dione-1,1-[1,8-dihydroxynaphthalene]-acetal (**3.79**, 2 mg, 0.006 mmol) was dissolved in anhydrous MeOH (1 mL) with cerium trichloride (3 mg, 0.012 mmol, 2 eq). The mixture was cooled in an ice-bath and $NaBH_4$ (0.3 mg, 0.008 mmol, 1.3 eq) was added. The mixture was stirred at 0 ºC for 1 hour, then $NaHCO_{3(aq)}$ (1M, 2 mL) was added. The mixture was extracted with diethyl ether (4 x 2 mL) and the combined phases dried over $MgSO_4$, filtered and the solvent was removed *in vacuo*. The residue was purified on deacidified silica using a step-wise gradient of 10 to 24% diethyl ether in petroleum ether. The product (**3.81**, 0.9 mg, 0.003 mmol, 42%) was obtained as a colourless oil.
R_f 0.21 (2:1 petroleum ether:ethyl acetate, visualization UV/PMA); IR (thin film) ν_{max} 1738, 1606, 1413, 1381, 1226 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.50 (1H, d, 8.3 Hz, H13 or H15), 7.47 (1H, d, 8.3 Hz, H13 or H15), 7.41 (1H, t, 7.2 Hz, H12 or H16), 7.40 (1H, t, 7.2 Hz, H12 or H16), 6.96 (1H, d, 7.6 Hz, H11 or H17), 6.87 (1H, d, 7.6 Hz, H11 or H17), 6.20 (1H, dd, 6.1 Hz, 1.8 Hz, H2 or H3), 6.12 (1H, d, 5.7 Hz, H6), 6.08 (1H, dd, 5.7 Hz, 2.3 Hz, H7), 6.05 (1H, dd, 6.1 Hz, 1.8 Hz, H2 or H3), 5.92 (1H, t, 1.8 Hz, H4), 4.81 (1H, dt, 7.0 Hz, 2.2 Hz, H8), 2.50 (1H, dd, 14.7 Hz, 7.2 Hz, H9), 2.25 (1H, 14.6 Hz,

2.6 Hz, H9), 2.14 (3H, s, H21); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.4 (C20), 148.3 (C, C10 or C18), 147.8 (C, C10 or C18), 137.7 (CH, C6 or C7), 137.4 (CH, C2 or C3), 134.3 (CH, C6 or C7), 134.1 (C, C14), 131.9 (CH, C2 or C3), 127.5 (CH, C12 or C16), 127.2 (CH, C12 or C16), 121.0 (CH, C13 or C15), 120.6 (CH, C13 or C15), 114.0 (C, C19), 109.20 (CH, C11 or C17), 109.17 (CH, C11 or C17), 109.0 (C, C1), 78.9 (C, C4), 75.9 (C, C8), 67.0 (C, C5), 35.5 (CH_2, C9), 21.1 (CH_3, C21); HRESIMS *m/z* = 333.1120 ± 1.4 ppm $[M+H]^+$ (333.1127 calcd for $C_{21}H_{16}O_4$).

Section 3.2.8

spiro-Nona-2,6-diene-1,4-dione-1,1-[1,8-dihydroxy-4-bromo-naphthalene]-acetal, 3.83 and _spiro_-nona-2,7-diene-1,4-dione-1,1-[1,8-dihydroxy-4-bromonaphthalene]-acetal, **3.84**

spiro-Nona-2,7-diene-1,4-dione-1,1-[1,8-dihydroxynaphthalene]-acetal (**3.34**, 5 mg, 17 μmol) was dissolved in CCl_4 (500 μL), N-bromosuccinimde (3.3 mg, 0.019 mmol, 1.1 eq) and AIBN (trace) were added and the reaction was stirred at room temperature for 20 hours. Additional N-bromosuccinimide (1.1 eq) was then added and the reaction stirred for a further 24 hours. After removal of the solvent *in vacuo*, the crude mixture was partially purified on deacidified silica using a stepwise gradient of petroleum ether to 6% diethyl ether in petroleum ether, giving a mixture of three brominated products. **3.83** (R_t 16.96 min, 0.6 mg, 1.6 μmol, 9%) was purified by separation on an analytical C_{18} HPLC column using 70% MeCN in H_2O (isocratic), then **3.84** (R_t 17.29 min, 0.9 mg, 2.4 μmol, 14%) and a minor product were separated on an analytical C_{18} HPLC column using 80% MeOH in H_2O (isocratic).

Mixture - R_f 0.54-0.63 (4:1 petroleum ether:ethyl acetate, visualization UV/PMA)

3.83: IR (thin film) ν_{max} 1729, 1609, 1413, 1365, 1269 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.80 (1H, dd, 8.6 Hz, 0.7 Hz, H15), 7.69 (1H, d, 8.1 Hz, H12), 7.56 (1H, dd, 8.6 Hz, 7.6 Hz, H16), 7.45 (1H, d, 6.1 Hz, H2), 7.01 (1H, dd, 7.6 Hz, 0.7 Hz, H17), 6.80 (1H, d, 8.1 Hz, H11), 6.47 (1H, d, 6.1 Hz, H3), 6.01 (1H, m, H7 or H8), 5.60 (1H, m, H7 or H8), 2.51 (2H, m, H6/9), 2.17 (2H, m, H6/9); ^{13}C NMR ($CDCl_3$, from 2D spectra at 500 MHz) δ 206.5 (C, C4), 153.7 (CH, C2), 147.8 (C, C18), 147.4 (C, C10), 137.0 (CH, C7 or C8),

136.2 (CH, C3), 133.0 (C, C14), 131.2 (CH, C12), 129.0 (CH, C16), 128.7 (CH, C7 or C8), 120.9 (CH, C15), 114.5 (C, C19), 110.6 (CH, C17), 110.5 (CH, C11), 69.9 (C, C5), 32.5 (CH_2, C6 or C9), 29.4 (CH_2, C6 or C9), C1/C13 not observed; HRESIMS *m/z* = 369.0115 $[M+H]^+$ 3.0 ppm (369.0126 calcd for $C_{19}H_{14}{}^{79}BrO_3$).

3.84: IR (thin film) ν_{max} 1732, 1609, 1413, 1365, 1268 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.81 (1H, dd, 8.6 Hz, 0.7 Hz, H15), 7.70 (1H, d, 8.1 Hz, H12), 7.56 (1H, dd, 8.5 Hz, 7.7 Hz, H16), 7.35 (1H, d, 6.1 Hz, H2), 7.01 (1H, dd, 7.7 Hz, 0.7 Hz, H17), 6.83 (1H, d, 8.1 Hz, H11), 6.41 (1H, d, 6.1 Hz, H3), 5.65 (2H, br s, H7/H8), 3.18 (2H, m, H6/H9), 2.63 (2H, m, H6'/H9'); ^{13}C NMR ($CDCl_3$, from 2D spectra at 500 MHz) δ 206.5 (C, C4), 152.9 (CH, C2), 147.6 (C, C18), 147.3 (C, C10), 135.9 (CH, C3), 132.8 (C, C14), 131.1 (CH, C12), 129.1 (CH, C16), 128.2 (CH, C7/C8), 121.0 (CH, C15), 114.3 (C, C19), 114.0 (C, C13), 110.8 (CH, C17), 110.5 (CH, C11), 106.4 (C, C1), 61.4 (C, C5), 38.3 (CH_2, C6/C9); HRESIMS *m/z* = 369.0131 $[M+H]^+$ 1.4 ppm (369.0126 calcd for $C_{19}H_{14}{}^{79}BrO_3$).

***spiro*-Nona-7-ene-1,4-dione-1,1-[1,8-dihydroxy-4-bromonaphthalene]-acetal, 3.85**

spiro-Nona-7-ene-1,4-dione-1,1-[1,8-dihydroxynaphthalene]-acetal (**3.35**, 4 mg, 0.014 mmol) was dissolved in CCl_4 (500 μL) and 1 M Br_2 solution (in CCl_4, 30 μL, 0.030, 2.2 eq) was added. The reaction was stirred for 30 minutes and partial decolourisation of the solution was observed. After removal of the solvent *in vacuo*, the crude product was purified on deacidified silica using a stepwise gradient from 0 to 3% diethyl ether in petroleum ether. This gave the product (**3.85**, 1.5 mg, 0.004 mmol, 29%) as a colourless oil. In addition, some starting material was recovered.

R_f 0.38 (4:1 petroleum ether:ethyl acetate, visualization UV/PMA); IR (thin film) ν_{max} 1750, 1608, 1412, 1365, 1269 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.78 (1H, dd, 8.5 Hz, 0.7 Hz, H15), 7.69 (1H, d, 8.1 Hz, H12), 7.54 (1H, dd, 8.5 Hz, 7.6 Hz, H16), 6.99 (1H, dd, 7.6 Hz, 0.7 Hz, H17), 6.80 (1H, d, 8.1 Hz, H11), 5.70 (2H, br s, H7/8), 3.01 (2H, m), 2.60 (2H, m), 2.54 (2H, t, 7.8 Hz), 2.17 (2H, t, 7.8 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 213.7 (C4), 147.6 (C10 or C18), 147.5 (C10 or C18), 132.6 (C14), 130.8 (C7, C8, C12 or C16), 128.7 (C7, C8, C12 or C16), 128.1 (C7, C8, C12 or C16), 128.0 (C7, C8, C12 or C16), 120.5 (C15), 114.6 (C19 or C13), 113.8 (C19 or C13), 110.5 (C11 or C17), 110.3 (C11 or

C17), 108.4 (C1), 61.9 (C5), 36.50 (C6 or C9), 36.47 (C6 or C9), 33.7 (C2 or C3), 28.1 (C2 or C3).

3,7,8-Tribromo-2-hydroxy-s*piro*-nona-2,7-diene-1,4-dione, 3.87 and 7,8-dibromo-2-hydroxy-s*piro*-nona-2,7-diene-1,4-dione, 3.88

spiro-Nona-7-ene-1,4-dione (**3.27**, 20 mg, 0.13 mmol) was dissolved in CCl_4 (500 μL) and a solution of bromine (1M, 150 μL, 0.15 mmol, 1.2 eq) was added. The solution decolourised rapidly and was stirred at room temperature for 5 hours. TLC/^{1}H NMR spectroscopy of the crude mixture at both 1 hour and 5 hours showed that largely starting material remained. Bromine (30 μL, 0.58 mmol, 4.5 eq) was added and stirred for 30 minutes, after which time TLC/^{1}H NMR spectroscopy of the crude mixture showed no starting material remained and a mixture of products had been formed. The solvent and residual bromine were removed *in vacuo* and the crude residue purified by chromatography on silica using 1 to 6% ether in petroleum ether to give 3,7,8-tribromo-2-hydroxy-s*piro*-nona-2,7-diene-1,4-dione (**3.87**, 21 mg, 0.05 mmol, 38%) as a slightly yellow crystalline solid and 7,8-dibromo-2-hydroxy-s*piro*-nona-2,7-diene-1,4-dione (**3.88**, 17 mg, 0.05 mmol, 38%) as a slightly yellow crystalline solid.

3.87: R_f 0.52 (4:1 petroleum ether:ethyl acetate, visualization UV/PMA); mp 163-164 ºC (recrystallised from diethyl ether); IR (diffuse reflectance) ν_{max} 2952, 1759, 1716, 1707, 1550, 1268, 1235, 1162 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.36 (2H, m, H7/8), 2.65 (2H, m, H6/9), 2.34 (2H, m, H6/9); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 194.2 (C2), 148.6 (C3), 56.7 (C5), 52.0 (C7/8), 42.0 (C6/9), C1/4 not observed; HRESIMS *m/z* = 398.7882 ± 3.8 ppm $[M-H]^-$ (398.7867 calcd for $C_9H_6{}^{79}Br_3O_3$).

3.88: R_f 0.38 (4:1 petroleum ether:ethyl acetate, visualization UV/PMA); mp 136-138 ºC (recrystallised from diethyl ether); IR (diffuse reflectance) ν_{max} 3059, 1758, 1707, 1560, 1275 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.54 (1H, br s, H3), 4.35 (2H, m, H7/8), 2.58 (2H, m, H6/9), 2.29 (2H, m, H6/9); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 200.0 (C4), 197.1 (C1), 148.4 (C3), 55.3 (C5), 52.0 (C7 or C8), 51.9 (C7 or C8), 41.6 (C6 or C9), 41.3 (C6 or C9) C2 not observed; HRESIMS *m/z* = 320.8775 ± 4.1 ppm $[M-H]^-$ (320.8762 calcd for $C_9H_7{}^{79}Br_2O_3$).

1,4-Hydroxy-*spiro*-nona-7-ene, 3.89 (stereoisomeric mixture)

spiro-Nona-7-ene-1,4-dione (**3.27**, 50 mg, 0.33 mmol) was dissolved in anhydrous MeOH (2 mL), $NaBH_4$ (33 mg, 0.84 mmol, 2.5 eq) was added and the reaction was stirred at room temperature for 2 hours. Citric acid (5 % w/v, 4 mL) was added and the reaction was extracted with ethyl acetate (3 x 4 mL). The combined organic phases were dried over $MgSO_4$, filtered and the solvents were removed *in vacuo*. This crude product (**3.89**, ~ 50 mg, ~ 100%), which was isolated as a colourless oil, was used directly in the next step. Analytical data for each diastereoisomer given below (**Section 3.2.8**).

IR (thin film) 3400-3200, 1417, 1344, 1095, 1053 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.74-5.58 (2H, m, H7/8), 4.40-3.75 (2H, m, H1/4), 2.68-2.56 (2H, m, H6 or H9), 2.10-1.93 (6H, m, H2/3/6/9).

1,4-Hydroxy-7,8-bromo-*spiro*-nonane, 3.90 (stereoisomeric mixture)

1,4-Hydroxy-*spiro*-nona-7-ene (**3.89**, ~50 mg, ~0.33 mmol) was dissolved in DCM (1 mL) and bromine (30 μL, 0.59 mmol, 1.8 eq) was added and the reaction stirred at room temperature for 2 hours. The solvent and excess bromine was removed *in vacuo* and this crude product (**3.90**, 100 mg, 0.32 mmol, ~ 100%), which was isolated as a yellowish oil, was used directly in the next step.

IR (thin film) 3400-3200, 1435, 1169, 1041 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 4.67-4.10 (4H, m, H1/4/7/8), 3.10-1.20 (8H, m, H2/3/6/9); HRFABMS m/z = 312.9417 $[M+H]^+$ 7.1 ppm (312.9439 calcd for $C_9H_{15}{}^{79}Br_2O_2$).

7,8-Dibromo-*spiro*-nona-1,4-dione, 3.86

1,4-Hydroxy-7,8-bromo-*spiro*-nonane (**3.90**, 100 mg, 0.32 mmol) was dissolved in DCM (3 mL), PDC (730 mg, 1.94 mmol, 6.1 eq) was added and the reaction was stirred at room temperature for 2 days. It was then filtered through a short silica pad using DCM and the solvent was removed *in vacuo* to give the product as an amorphous white solid (**3.86**, 50 mg, 0.16 mmol, 50%).

mp 127-132 ºC (recrystallised from diethyl ether); IR (diffuse reflectance) ν_{max} 1718, 1415, 1281, 1176 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 4.27 (2H, m, H7/8), 2.85 (4H, m, H2/3), 2.58 (2H, m, H6/9), 2.23 (2H, m, H6/9); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 212.6

(C1/4), 61.1 (C5), 52.6 (C7/8), 41.9 (C2/3), 35.2 (C6/9); HRESIMS *m/z* = 306.8972 ± 0.7 ppm $[M+H]^+$ (306.8970 calcd for $C_9H_9{}^{79}Br_2O_2$).

7,8-Dibromo-*spiro*-nona-1,4-dione-1,1,4,4-di[ethane-1,2-diol]-acetal, 3.91

7,8-Dibromo-*spiro*-nona-1,4-dione (**3.86**, 20 mg, 0.065 mmol) was dissolved in benzene (3 mL), ethylene glycol (400 μL, 7.2 mmol, 111 eq) and pTSA (1 mg, 0.005 mmol, 8 mol%) were added and the reaction was refluxed for 16 hours with a Dean-Stark apparatus for H_2O removal. Additional ethylene glycol (200 μL, 3.6 mmol, 55 eq) was added to the reaction and refluxing was continued for a further 5 hours, then the reaction was cooled to room temperature. Diethyl ether (10 mL) was added and the reaction was washed with $NaHCO_{3(aq)}$ (1M, 4 x 5 mL). The organic phase was dried over $MgSO_4$, filtered and the solvents removed *in vacuo* to give the product (**3.91**, 26 mg, 0.065 mmol, ~ 100%) as a colourless oil.

R_f 0.13 (4:1 petroleum ether:ethyl acetate, visualization UV/PMA); IR (thin film) ν_{max} 1443, 1322, 1168, 1141, 1053 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 4.12 (2H, m, H7/8), 4.02-3.94 (8H, m, H10/11/12/13), 2.49 (2H, dd, 14.0 Hz, 6.7 Hz, H6/9), 2.17 (2H, m, H6/9), 1.93-1.86 (4H, m, H2/3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 115.7 (C, C1/4), 65.0 (CH_2, C10/11/12/13), 57.7 (C, C5), 54.6 (CH, C7/8), 38.1 (CH_2, C6/9), 32.1 (CH_2, C2 or C3), 31.9 (CH_2, C2 or C3); HRESIMS *m/z* = 396.9658 ± 2.0 ppm $[M+H]^+$ (396.9650 calcd for $C_{13}H_{19}{}^{79}Br_2O_4$).

7-Bromo-*spiro*-nona-7-ene-1,4-dione-1,1,4,4-di[ethane-1,2-diol]-acetal, 3.93

Dibrominated **3.91** (15 mg, 0.038 mmol) was dissolved in a solution of ^{t}BuOK in anhydrous THF (1 mL, 1 mmol, 26 eq) and the reaction was stirred at room temperature for 16 hours. After removal of the solvent *in vacuo*, the crude residue was repeatedly partitioned between diethyl ether (3 x 2 mL) and H_2O (2 mL). The organic phase was dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. The crude residue was purified by chromatography on silica using 0 to 20% ether in petroleum ether.

R_f 0.18 (4:1 petroleum ether:ethyl acetate, visualization UV/PMA); IR (thin film) ν_{max} 1321, 1147, 1045 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.73 (1H, quintet, 2.2 Hz, H8), 4.01-3.92 (8H, m, OC**H**$_2$C**H**$_2$O), 2.76 (2H, q, 2.2 Hz, H6 or H9), 2.52 (2H, q, 2.3 Hz, H6

or H9), 1.93 (4H, s, H2/3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 129.2 (CH, C8), 118.0 (C, C1/4), 116.4 (C, C7), 65.2 (CH_2, O**C**H_2**C**H_2O), 65.1 (CH_2, O**C**H_2**C**H_2O), 59.1 (C, C5), 42.4 (CH_2, C6 or C9), 36.3 (CH_2, C6 or C9), 32.3 (CH_2, C2 and C3); HRESIMS *m/z* = 317.0377 ± 3.5 ppm $[M+H]^+$ (317.0388 calcd for $C_{13}H_{18}{}^{79}BrO_4$).

Section 3.2.9

_O_-Acetyl-4,8-dihydroxy-*spiro*-nona-2,6-diene-1-one-1,1-[1,8-dihydroxynaphthalene]-acetal, 3.94 (stereoisomeric mixture)

A mixture of *O*-acetyl-4-hydroxy-*spiro*-nona-2,6-diene-1,8-dione-1,1-[1,8-dihydroxynaphthalene]-acetal and *O*-acetyl-4-hydroxy-*spiro*-nona-2,8-diene-1,6-dione-1,1-[1,8-dihydroxynaphthalene]-acetal (**3.79** and **3.80**, 12 mg, 0.034 mmol) were dissolved in anhydrous MeOH (1 mL) with cerium trichloride (10 mg, 0.041 mmol, 1.2 eq). The mixture was cooled in an ice-bath and sodium borohydride (1.3 mg, 0.034 mmol, 1 eq) was added. The mixture was stirred at 0 ºC for 1 hour, then $NaHCO_{3(aq)}$ (1M, 3 mL) was added. The mixture was extracted with diethyl ether (4 x 3 mL) and the combined phases dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. The residue was purified on deacidified silica using a step-wise gradient of 10 to 24% diethyl ether in petroleum ether. The product (**3.94**, 11 mg, 0.031 mmol, 91%) was obtained as a mixture of stereoisomers as a colourless oil.

IR (thin film) ν_{max} 3400-3100, 2916, 1729, 1719, 1607, 1412, 1379 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.51-7.36 (4H, m, H12, H13, H15 and H16), 6.98-6.82 (2H, m, H11 and H17), 6.22-5.75 (5H, m, H2, H3, H4, H6 and H7), 4.84-4.79 (1H, m, H8), 3.52-1.77 (2H, m, H9), 2.14 (~2H, s, H21), 2.10 (~1H, s, H21); HRESIMS *m/z* = 333.1124 ± 0.9 ppm $[M-OH]^+$ (333.1127 calcd for $C_{21}H_{16}O_4$).

Burgess reagent - methyl (carboxysulfamoyl)triethylammonium hydroxide, 3.96 [21]

Step 1, methyl (chlorosulfonyl)carbamate

Freshly distilled chlorosulfonyl isocyanate (2 mL, 26.6 mmol, 1.16 eq) was dissolved in anhydrous benzene (7 mL) and placed in a H_2O bath. Anhydrous MeOH (930 μL, 23.0 mmol), was added over 30 minutes. Petroleum ether (6 mL) was added and the mixture placed in an ice-bath for 30 minutes. The white precipitate formed was filtered and washed with petroleum ether (2 x 10 mL) and then dried *in vacuo*. The product was obtained as white amorphous solid (3.23 g, 18.6 mmol, 81%).

mp 71-74 ºC (lit. 72-74 ºC).[21]

Step 2, methyl (carboxysulfamoyl)triethylammonium hydroxide, **3.96**

Anhydrous TEA (3.6 mL, 25.8 mmol, 2.2 eq) was dissolved in anhydrous benzene (10 mL) and placed in a H_2O bath. Methyl (chlorosulfonyl)carbamate (2 g, 11.5 mmol) was dissolved in anhydrous benzene (25 mL) and this mixture was added to the TEA solution over 30 minutes. The mixture was stirred at room temperature for a further hour. The white precipitate formed was filtered and the filtrate reduced *in vacuo*. Anhydrous THF (18 mL) was added to the residue and the solution cooled to -20 ºC. After 1 hour, crystallisation was observed and after 16 hours, the product (**3.96**, white crystals, 1.73 g, 7.25 mmol, 63%) was collected by filtration and dried *in vacuo*.

mp 72-75 ºC (lit. 70-72 ºC)[21]

***O*-Acetyl-4-hydroxy-*spiro*-nona-2,6,8-triene-1,1-[1,8-dihydroxynaphthalene]-acetal, 3.95**

Allyl alcohol **3.94** (mixture of isomers, 11 mg, 0.031 mmol) was dissolved in anhydrous benzene (2 mL) and Burgess reagent (**3.96**, 15 mg, 0.063 mmol, 2 eq) was added. The reaction was stirred at 50 ºC for 16 hours, then cooled to room temperature. Diethyl ether (3 mL) was added and the mixture was extracted with $NaHCO_{3(aq)}$ (1M, 2 x 3 mL). The organic phase was dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. The crude residue was purified by chromatography on deacidified silica using a step-wise gradient of 0 to 6% diethyl ether in petroleum ether, which gave the product (**3.95**, 3mg, 0.009 mmol, 29%) as a colourless oil.

R_f 0.41 (4:1 petroleum ether:ethyl acetate, visualization UV/PMA); IR (thin film) ν_{max} 1739, 1607, 1412, 1380, 1274, 1233, 1222, 1025 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.42 (1H, d, 8.4 Hz, H13 or H15), 7.41 (1H, d, 8.4 Hz, H13 or H15), 7.37-7.33 (2H, m, H12 and H16), 6.85 (1H, dd, 7.6 Hz, 0.9 Hz, C11 or C17), 6.80 (1H, dd, 7.6 Hz, 0.9 Hz, H11 or C17), 6.38 (1H, dd, 5.9 Hz, 2.2 Hz, H3), 6.31 (1H, ddd, 5.3 Hz, 2.2 Hz, 1.5 Hz, H6, H7, H8 or H9), 6.29-6.26 (2H, m, H2 and H6, H7 H8 or H9), 6.18-6.14 (2H, m, H6, H7 H8 or H9), 6.00 (1H, dd, 2.2 Hz, 1.1 Hz, H4), 2.03 (3H, s, H21); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.3 (C, C20), 148.3 (C, C10 or C19), 148.1 (C, C10 or C19), 136.4 (CH, C3), 135.9 (CH, C6, C7, C8 or C9), 134.4 (CH, C2, C6, C7, C8 or C9), 134.3 (CH, C2, C6, C7, C8 or C9), 132.6 (CH, C6, C7, C8 or C9), 132.2 (CH, C6, C7, C8 or C9), 127.1 (CH, C12 and C16), 120.4 (CH, C13 or C15), 120.3 (C13 or C15), 113.9 (C, C19), 111.6 (C, C1), 108.8 (CH, C11 or C18), 108.7 (CH, C11 or C18), 78.3 (CH, C4), 72.8 (C, C5), 21.0 (CH_3, C21); HRESIMS *m/z* = 333.1117 ± 3.0 ppm $[M+H]^+$ (333.1127 calcd for $C_{21}H_{16}O_4$).

Section 3.2.9

_O_-Acetyl-4-hydroxy-*spiro*-nona-2,6-diene-8,9-dioxetane-1,1-[1,8-dihydroxynaphthalene]-acetal, 3.98

O-Acetyl-4-hydroxy-*spiro*-nona-2,6,8-triene-1,1-[1,8-dihydroxynaphthalene]-acetal (1 mg, 0.003 mmol) was dissolved in DCM (0.5 mL) and MeOH (0.5 mL) and thiourea (0.2 mg, ~0.003 mmol, ~1 eq) and Rose Bengal (trace) were added. The mixture was placed under an atmosphere of oxygen at atmospheric pressure and cooled in an ice-bath. A 150 W tungsten lamp was used as the light source. The reaction was vigorously stirred in an ice-bath for 2 hours, then warmed to room temperature for 16 hours. Ethyl acetate (4 mL) was added and the organic phase was extracted with $NaHCO_{3(aq)}$ (1M, 3 x 3 mL). The organic phase was dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. The residue was purified by chromatography by passing it through an analytical HPLC C_{18} column in 5 aliquots using the following elution conditions [solvents: A: H_2O + 0.05% TFA, B: MeCN; isocratic: 0 min 10%, linear gradient: 2 min 10% B, 14 min 75% B, isocratic 24 min 75%, linear gradient: 35 min 100% B; 40 ºC; 1 mL min^{-1}, detection at

330 nm]. This gave a partially purified mixture of two products (R_t 13.0 mins and 13.8 mins) which could be separated by passing the mixture through an analytical HPLC C_{18} column in 5 aliquots using the following elution conditions [solvents: A: H_2O + 0.05% TFA, B: MeOH; isocratic: 0 min 10%, linear gradient: 2 min 10% B, 14 min 75% B, isocratic 24 min 75%, linear gradient: 35 min 100% B; 40 ºC; 1 mL min^{-1}, detection at 330 nm]. The first product (R_t 16.8 mins) could not be obtained in sufficient quantity for full elucidation of its structure, however it was clearly related to the second product (**3.98**, R_t 18,2 mins, ~0.3 mg, ~0.0008 mmol, ~27%), which was obtained as an amorphous solid and was assigned as *O*-acetyl-4,8,9-hydroxy-*spiro*-nona-2,6-diene-1,1-[1,8-dihydroxynaphthalene]-acetal.

^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.55 (2H, d, 8.4 Hz, H13 and H15), 7.46 (1H, t, 8.2 Hz, H12 or H16), 7.45 (1H, t, 8.2 Hz, H12 or H16), 6.97 (2H, d, 7.7 Hz, H11 or H17), 6.29 (1H, dd, 6.2 Hz, 1.8 Hz, H3), 6.26 (1H, t, 1.8 Hz, H4), 6.16 (1H, dd, 6.3 Hz, 1.7 Hz, H6 or H7), 6.10 (1H, dd, 6.2 Hz, 1.8 Hz, H2), 5.88 (1H, dd, 6.3 Hz, 1.4 Hz, H6 or H7), 5.48 (1H, dt, 7.2 Hz, 1.6 Hz, H8), 3.86 (1H, d, 7.2 Hz, C9), 2.07 (3H, s, H21); ^{13}C NMR ($CDCl_3$, 500 MHz, observed by 2D NMR) δ 171.6 (C, C20), 136.8 (CH, C3), 133.6 (CH, C6 or C7), 132.2 (CH, C6 or C7), 130.0 (CH, C2), 127.4 (CH, C12 and C16), 121.0 (CH, C13 and C15), 109.3 (CH, C11 and C18), 86.0 (CH, C8), 78.0 (CH, C4), 65.0 (CH, C9), 19.4 (CH_3, C21); HREIMS m/z = 364.0935 $[M]^+$ 3.3 ppm (364.0947 calcd for $C_{21}H_{16}O_6$).

trans-1,4-Dihydroxy-_spiro_-nona-7-ene, *trans*-**3.89**

A crude mixture of *cis*- and *trans*-**3.89** was prepared as above (**Section 3.2.7**) from **3.27** (200 mg, 1.33 mmol). The mixture was then purified by chromatography on silica using a stepwise gradient of 10 – 50% diethyl ether in petroleum ether. *cis*-1,4-Dihydroxy-*spiro*-nona-7-ene (*cis*-**3.89**, 103 mg, 0.68 mmol, 51%) and *trans*-1,4-dihydroxy-*spiro*-nona-7-ene (*trans*-**3.89**, 87 mg, 0.57 mmol, 43%) were obtained as colourless oils.

cis-**3.89**: R_f 0.35 (1:1 petroleum ether:ethyl acetate, visualization UV/PMA); IR (thin film) ν_{max} 3500-3100, 1456-1420, 1173, 1053, 1038 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.76-5.72 (1H, m, H7 or H8), 5.58 (1H, m, H7 or H8), 3.88 (2H, br s, H1/4), 2.66 (2H, m, H6/9), 2.05 (2H, m, H2/3), 1.94 (4H, m, H2/3/6/9); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 130.4

(CH, C7 or C8), 127.8 (CH, C7 or C8), 80.4 (CH, C1/4), 41.7 (CH_2, C6 or C9), 33.6 (CH_2, C6 or 9), 31.8 (CH_2, C2/3).

trans-**3.89**: R_f 0.16 (1:1 petroleum ether:ethyl acetate, visualization UV/PMA); IR (thin film) ν_{max} 3500-3100, 1622, 1455-1420, 1173, 1053, 1037 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.68 (2H, br s, H7/8), 4.07 (2H, m, H1/4), 2.64 (2H, br d, 14.8 Hz, H6/9), 2.13 (2H, m, H2/3), 2.04 (2H, br d, 14.8 Hz, H6/9), 1.54 (2H, m, H2/3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 129.4 (CH, C7/8), 78.2 (CH, C1/4), 56.9 (C, C5), 36.5 (CH_2, C6/9), 29.6 (CH_2, C2/3); HRCIMS *m*/*z* = 172.1336 $[M+NH_4]^+$ 0.7 ppm (172.1338 calcd for $C_9H_{18}NO_2$).

_O,O_-Diacetyl-_trans_-1,4-dihydroxy-_spiro_-nona-7-ene, **3.99**

trans-1,4-Dihydroxy-*spiro*-nona-7-ene (*trans*-**3.89**, 87 mg, 0.56 mmol) was dissolved in anhydrous pyridine (4 mL) and acetic anhydride (1.5 mL, 15.9 mmol, 28 eq) and the mixture was stirred at room temperature for 16 hours. Citric $acid_{aq}$ (5%, 10 mL) was added and the mixture extracted with diethyl ether (3 x 10 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered and reduced *in vacuo* to give the product as a colourless oil (**3.99**, 100 mg, 0.42 mmol, 75%).

IR (thin film) ν_{max} 1739, 1374, 1240, 1028 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.58 (2H, br s, H7/H8), 5.04 (2H, m, H1/H4), 2.55 (2H, br d, 14.9 Hz, H6 and H9), 2.25-2.17 (2H, m, H2 and H3), 2.11 (2H, br d, 14.9 Hz, H6 and H9), 2.03 (6H, s, H11 and H13), 1.61-1.54 (2H, m, H2 and H3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.2 (C, C10/12), 129.0 (CH, C7/8), 79.6 (CH, C1/4), 55.1 (C, C5), 37.2 (CH_2, C6/9), 27.6 (CH_2, C2/3), 21.5 (CH_3, C11/13); HRDCIMS *m*/*z* = 239.1279 $[M+H]^+$ 1.8 ppm (239.1283 calcd for $C_{13}H_9O_4$).

_O,O_-Diacetyl-_trans_-1,4-dihydroxy-_spiro_-nona-6-ene-8-one, 3.100

O,O-Diacetyl-*trans*-1,4-dihydroxy-*spiro*-nona-7-ene (**3.99**, 100 mg, 0.42 mmol) was dissolved in anhydrous DCM, and $NaHCO_3$ (17 mg, 0.21 mmol, 0.5 eq) and dirhodium(II)tetrakis(caprolactam) (2.6 mg, 0.003 mmol, 1 mol%) were added. ^{t}Butyl hydroperoxide 5M in decanes (2 mL, 10 mmol, 24 eq) was added and an immediate colour change from light purple to deep pink was observed. The reaction was stirred at room temperature for 16 hours, then purified on silica (deacidified) using a stepwise

gradient of 10 to 40% diethyl ether in petroleum ether to give the product as a colourless oil (**3.100**, 84 mg, 0.33 mmol, 79%).

R_f 0.35 (1:1 petroleum ether:ethyl acetate, visualization PMA); IR (thin film) ν_{max} 1742, 1717, 1375, 1236, 1030 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.50 (1H, d, 5.8 Hz, H6), 6.23 (1H, d, 5.8 Hz, H7), 5.39 (1H, t, 7.7 Hz, H1 or H4), 5.04 (1H, dd, 6.1 Hz, 2.2 Hz, H1 or H4), 2.63 (1H, d, 18.2 Hz, H9), 2.39 (1H, m, H2 or H3), 2.33-2.24 (1H, m, H2 or H3), 2.09 (3H, s, H11 or H13), 2.05 (1H, d, 18.4 Hz, H9), 2.01 (3H, s, H11 or H13), H 1.81 (1H, m, H2 or H3), 1.74-1.62 (1H, m, H2 or H3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 206.6, 170.0, 163.4, 135.7, 80.6, (79.0), (76.2), 58.1, 39.8, 28.3, 27.6, 21.0, 20.9; HRESIMS *m/z* = 253.1073 ± 1.2 ppm $[M+H]^+$ (253. 1076 calcd for $C_{13}H_{17}O_5$).

_O,O_-Diacetyl-_trans_-1,4,8-trihydroxy-_spiro_-nona-6-ene, 3.101

O,O-Diacetyl-*trans*-1,4-dihydroxy-*spiro*-nona-6-ene-8-one (**3.100**, 84 mg, 0.33 mmol) was dissolved in anhydrous MeOH (1 mL), cerium trichloride (anhydrous, 99 mg, 0.40 mmol, 1.2 eq) was added and the mixture was cooled in an ice-bath. Sodium borohydride (13 mg, 0.33 mmol, 1 eq) was added and the reaction was stirred at 0 ºC for 1 hour. Deionised H_2O (3 mL) was added and the reaction extracted with diethyl ether (4 x 3 mL). The combined organic phases were dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. The crude product was purified by chromatography on deacidified silica using a stepwise gradient of petroleum ether 20 to 50% diethyl ether in petroleum ether, to give the product (**3.101**, mixture of isomers, 74 mg, 0.29 mmol, 88%) as a colourless oil.

R_f 0.33 (1:1 petroleum ether:ethyl acetate, visualization PMA); IR (thin film) ν_{max} 3400-3200, 1740, 1375, 1238, 1028 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.99 (1/2H, dd, 5.6 Hz, 2.3 Hz, H7), 5.95 (1/2H, dd, 5.6 Hz, 2.2 Hz, H7), 5.75 (1/2H, d, 5.7 Hz, H6), 5.73 (1/2H, dd, 5.7 Hz, 1.0 Hz, H6), 5.14 (1/2H, t, 7.6 Hz, H1 or H4), 5.10 (1/2H, t, 6.4 Hz, H1 or H4), 5.04 (1/2H, m, H1 or H4), 4.88 (1/2H, m, H1 or H4), 4.82-4.74 (1H, m, H8), 2.47-2.17 (3H, m, H2, H3 and H9), 2.08 (3/2H, s), 2.06 (3/2H, s), 2.04 (3/2H, s), 2.03 (3/2H, s), 1.97-1.51 (3H, m, H2, H3 and H9); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.9 (C, C10 or C12), 170.6 (C, C10 or C12), 170.4 (C, C10 or C12), 170.2 (C, C10 or C12), 136.7 (CH, C7),136.6 (CH, C7), 134.7 (CH, C6), 134.6 (CH, C6), 81.3 (CH, C1 or C4), 81.1 (CH, C1 or C4), 78.1 (CH, C1 or C4), 77.5 (CH, C1 or C4), 76.5 (CH, C1 or C4), 61.8 (C, C5),

61.5 (C, C5), 39.6 (CH_2, C2, C3 or C9), 38.9 (CH_2, C2, C3 or C9), 28.04 (CH_2, C2, C3 or C9), 28.01 (CH_2, C2, C3 or C9), 27.9 (CH_2, C2, C3 or C9), 27.3 (CH_2, C2, C3 or C9), 21.1 (CH_3, C11/13); HRESIMS *m/z* = 237.1118 ± 3.8 ppm $[M\text{-}OH]^+$ (237.1127 calcd for $C_{13}H_{17}O_4$).

_O,O_-Diacetyl-_trans_-1,4,-dihydroxy-_spiro_-nona-6,8-diene, 3.102

O,O-Diacetyl-*trans*-1,4,8-trihydroxy-*spiro*-nona-6-ene (**3.101**, 74 mg, 0.29 mmol) was dissolved in anhydrous benzene (3 mL) and Burgess reagent (**3.96**, 21 mg, 0.87 mmol, 3 eq) was added. The reaction was warmed to 50 ºC for 7 hours, then cooled to room temperature. Deionised H_2O (3 mL) was added and the mixture was extracted with diethyl ether (4 x 2 mL). The combined organic phases were dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. The crude product was purified by chromatography on deacidified silica using a stepwise gradient of 0 to 12% diethyl ether in petroleum ether, to give the product as a colourless oil (**3.102**, ~8 mg, ~0.034 mmol, ~ 12%). A complex mixture of the isomers of **3.103** was obtained on elution with diethyl ether. R_f 0.38 (4:1 petroleum ether:ethyl acetate, visualization UV/PMA); IR (thin film) ν_{max} 1738, 1373, 1232, 1026 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.38 (2H, m, H7/H8), 6.25 (2H, m, H6/H9), 5.36 (2H, m, H1/H4), 2.47 (2H, m, H2 and H3), 1.99 (6H, s, H11/H13), 1.87 (2H, m, H2 and H3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.4, 135.4, 133.0, 69.5, 29.8, 21.1; HRESIMS *m/z* = 237.1136 ± 3.8 ppm $[M+H]^+$ (237.1127 calcd for $C_{13}H_{17}O_4$).

Retro Diels-Alder reaction of 3.103, giving 3.102

The stereoisomeric mixture of **3.102** dimers, **3.103** (~ 50 mg, ~ 0.11 mmol), was dissolved in anhydrous benzene (30 mL) and the reaction refluxed for 3 days. After this time ^1H NMR spectroscopy of the crude mixture showed no more dimer was present and the monomer, **3.102**, had been formed. After cooling to room temperature and removal of the solvent *in vacuo*, the crude residue was purified by chromatography on deacidified silica as above. The monomer **3.103** was obtained (4 mg, 0.02 mmol, ~18%).

Section 3.3

_O_-Acetyl-4-hydroxy-_spiro_-nona-7-ene-1-one-1,1-[1,8-dihydroxynaphthalene]-acetal (or _O_-acetyl-2,3-dihydro-6,9-deoxy-_spiro_-mamakone A), 3.104

4-Hydroxy-s*piro*-nona-7-ene-1-one-1,1-[1,8-dihydroxynaphthalene]-acetal (20 mg, 0.068 mmol) was dissolved in anhydrous pyridine (500 μL) and acetic anhydride was added (200 μL, 2.1 mmol, 31 eq). The reaction was stirred at room temperature for 16 hours. Deionised H_2O (3 mL) was added and the mixture extracted with diethyl ether (4 x 3 mL). The combined organic phases were dried over $MgSO_4$, filtered and reduced *in vacuo*. No further purification was required. The product (**3.104**, 19 mg, 0.056 mmol, 82 %) was obtained as a colourless oil.

IR (thin film) ν_{max} 1738, 1609, 1412, 1381, 1243 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.45-7.36 (4H, m, H12/H13/H15/H16), 6.90 (1H, d, 7.5 Hz, H11 or H17), 6.88 (1H, d, 7.5 Hz, H11 or H17), 5.71-5.65 (2H, m, H7 and H8), 5.41 (1H, dd, 8.2 Hz, 6.7 Hz, H4), 3.05 (1H, m, H6 or H9), 2.85 (1H, m, H6 or H9), 2.68 (1H, m, H6 or H9), 2.42 (1H, m, H6 or H9), 2.28 (1H, m, H3), 2.09 (3H, s, H21), 1.93 (2H, m, H2), 1.72 (1H, m, H3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.3 (C, C20), 148.5 (C, C10 or C18), 148.4 (C, C10 or C18), 134.5 (C, C14), 129.01 (CH, C7 or C8), 128.97 (CH, C7 or C8), 127.6 (CH, C12 or C16), 127.5 (CH, C12 or C16), 120.6 (CH, C13 or C15), 120.5 (CH, C13 or C15), 114.4 (C, C19), 110.0 (C, C1), 109.7 (CH, C11 or C18), 109.4 (CH, C11 or C18), 79.3 (CH, C4), 57.8 (C, C5), 38.2 (CH_2, C6 or C9), 34.4 (CH_2, C6 or C9), 30.9 (CH_2, C2), 26.3 (CH_2, C3), 21.5 (C21); HRESMS *m*/*z* = 336.1366 $[M]^{+\cdot}$ 1.2 ppm (336.1362 calcd for $C_{21}H_{20}O_4$).

_O_-Acetyl-4-hydroxy-_spiro_-nona-6-ene-1,8-dione-1,1-[1,8-dihydroxynaphthalene]-acetal, 3.105 ([4*R*,5*R*] and [4*S*,5*S*]) and 3.106 ([4*R*,5*S*] and [4*S*,5*R*])

O-Acetyl-4-hydroxy-*spiro*-nona-7-ene-1-one-1,1-[1,8-dihydroxynaphthalene]-acetal (**3.104**, 19 mg, 0.056 mmol) was dissolved in DCM (1 mL) and dirhodium(II)tetrakis(caprolactam) (0.4 mg, 0.56 μmol, 1 mol%) and $NaHCO_3$ (2.3 mg, 0.028 mmol, 0.5 eq) were added. ^{t}Butyl hydroperoxide (5M in decanes, 0.5 mL, 2.5 mmol, 45 eq) was added and the reaction stirred at room temperature for 16 hours. The solvents were removed *in vacuo*, and the crude residue purified chromatography on silica (deacidified) using a step-wise gradient of 0 to 20% diethyl ether in petroleum ether. The

isomeric products *O*-acetyl-4-hydroxy-*spiro*-nona-6-ene-1,8-dione-1,1-[1,8-dihydroxynaphthalene]-acetal (**3.105**, 8.7 mg, 0.025 mmol, 45%) and *O*-acetyl-4-hydroxy-*spiro*-nona-8-ene-1,7-dione-1,1-[1,8-dihydroxynaphthalene]-acetal (**3.106**, 4.1 mg, 0.012 mmol, 21%) were obtained as colourless oils.

3.105: R_f 0.19 (4:1 petroleum ether:ethyl acetate, visualization PMA); IR (thin film) ν_{max} 1741, 1721, 1610, 1411, 1380, 1276, 1236, 1053 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.67 (1H, d, 5.8 Hz, H6), 7.49-7.36 (4H, m, H12, H13, H15 and H16), 6.97 (1H, d, 7.4 Hz, H11 or H17), 6.86 (1H, d, 7.4 Hz, H11 or H17), 6.18 (1H, d, 5.8 Hz, H7), 5.72 (1H, t, 8.5 Hz, H4), 2.86 (1H, d, 18.1 Hz, H9), 2.66 (1H, d, 18.1 Hz, H9), 2.43 (1H, m, H3), 2.16 (1H, m, H2), 2.05 (1H, m, H2), 2.04 (3H, s, H21), 1.82 (1H, m, H3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 207.4 (C, C8), 170.7 (C, C20), 162.6 (CH, C6), 147.6 (C, C10 or C18), 147.4 (C, C10 or C18), 136.5 (C, C14), 134.5 (CH, C7), 127.7 (CH, C12 or C16), 127.6 (CH, C12 or C16), 121.2 (CH, C13 or C15), 121.1 (CH, C13 or C15), 114.0 (C, C19), 109.8 (CH, C11 or C17), 109.6 (CH, C11 or C17), 108.9 (C, C1), 74.9 (CH, C4), 61.7 (C, C5), 36.8 (CH_2, C9), 32.1 (CH_2, C2), 26.8 (CH_2, C3), 21.2 (CH_3, C21); HRESIMS *m/z* = 351.1235 ± 0.9 ppm $[M+H]^+$ (351.1232 calcd for $C_{21}H_{19}O_5$).

3.106: R_f 0.14 (4:1 petroleum ether:ethyl acetate, visualization PMA); IR (thin film) ν_{max} 1738, 1721, 1609, 1412, 1379, 1276, 1236, 1054 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.65 (1H, d, 5.9 Hz, H9), 7.48 (2H, d, 8.1 Hz, H13 and H15), 7.41 (1H, t, 7.4 Hz, H12 or H16), 7.39 (1H, t, 7.4 Hz, H12 or H16), 6.91 (1H, d, 7.5 Hz, H11 or H17), 6.87 (1H, d, 7.5 Hz, H11 or H17), 6.31 (1H, d, 5.8 Hz, H8), 5.46 (1H, dd, 8.1 Hz, 6.3 Hz, H4), 3.06 (1H, d, 18.9 Hz, H6), 2.45 (1H, mH3), 2.43 (1H, d, 18.8 Hz, H6), 2.21 (1H, m, H2), 2.10 (1H, m, H2),. 2.09 (3H, s, H21), 1.95 (1H, m, H3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 207.5 (C, C6), 170.7 (C, C20), 160.6 (CH, C9), 147.8 (C, C10 or C18), 147.5 (C, C10 or C18), 136.2 (C, C14), 134.5 (C, C8), 127.7 (CH, C12 or C16), 127.6 (CH, C12 or C16), 121.2 (CH, C13 or C15), 121.1 (CH, C13 or C15), 114.0 (C, C19), 109.9 (CH, C11 or C17), 109.6 (CH, C11 or C17), 108.9 (C, C1), 78.8 (CH, C4), 61.7 (C, C5), 40.7 (CH_2, C9), 31.9 (CH_2, C2), 27.3 (CH_2, C3), 21.2 (CH_3, C21); HRESIMS *m/z* = 351.1242 ± 2.8 ppm $[M+H]^+$ (351.1232 calcd for $C_{21}H_{19}O_5$).

4,8-Dihydroxy-*spiro*-nona-2,6-diene-1-one-1,1-[1,8-dihydroxynaphthalene]-acetal, 3.107 (mixture of diastereoisomers)

spiro-Nona-2,7-diene-1,4,6-trione-1,1-[1,8-dihydroxynaphthalene]-acetal (**3.77**, 3.2 mg, 0.010 mmol) and $CeCl_3$ (anhydrous, 5.4 mg, 0.022 mmol, 2.1 eq) were dissolved in MeOH (500 μL) and cooled to 0 ºC. $NaBH_4$ (0.8 mg, 0.022 mmol, 2.1 eq) was added and the reaction stirred at 0 ºC for 1 hour. $NaHCO_{3\ (aq)}$ 1 M (1 mL) was added and the reaction extracted with diethyl ether (4 x 1 mL). The combined organic phases were dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. The crude product, obtained as a colourless oil (**3.107**, mixture of isomers, 3.2 mg, 0.010 mmol, 98%), was used directly in the next step.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.55-7.34 (4H, H12/13/15/16), 7.0-6.82 (2H, H11/17), 6.38-5.94 (4H, H2/3/7/8), 4.98-4.62 (2H, H4/6), 2.91-2.63 (1H, H9), 2.32-1.96 (1H, H9); HRESIMS *m/z* = 331.0961 $[M+Na]^+$ 4.5 ppm (331.0946 calcd for $C_{19}H_{16}O_4Na$).

***O,O*-Diacetyl-4,8-dihydroxy-*spiro*-nona-2,6-diene-1-one-1,1-[1,8-dihydroxynaphthalene]-acetal, 3.108**

4,8-Dihydroxy-*spiro*-nona-2,6-diene-1-one-1,1-[1,8-dihydroxynaphthalene]-acetal (**3.107**, 3.2 mg, 0.010 mmol) was dissolved in pyridine (500 μL) and acetic anhydride was added (200 μL). The reaction was stirred at room temperature for 16 hours, then diethyl ether was added (2 mL) and the mixture extracted with $NaHCO_{3\ (aq)}$ (1M, 3 x 2 mL). After drying over $MgSO_4$, filtration and evaporation of solvent, the crude product was purified on silica (deacidified) using a stepwise gradient of 0 to 12% diethyl ether in petroleum ether, to give the product (mixture of isomers, 3.1 mg, 79%) as a colourless oil.

R_f 0.35 (4:1 petroleum ether:ethyl acetate, visualization UV/PMA);

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.49-7.35 (4H, H12/13/15/16), 6.95-6.82 (2H, H11/17), 6.22-5.72 (6H, H2/3/4/6/7/8), 3.15-2.41 (2H, H9), 2.17-2.04 (6H, H21/23); HRESIMS *m/z* = 415.1167 $[M+Na]^+$ 2.2 ppm (415.1158 calcd for $C_{23}H_{20}O_6Na$).

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JOC Article

Pteratides I−IV, New Cytotoxic Cyclodepsipeptides from the Malaysian Basidiomycete *Pterula* sp.

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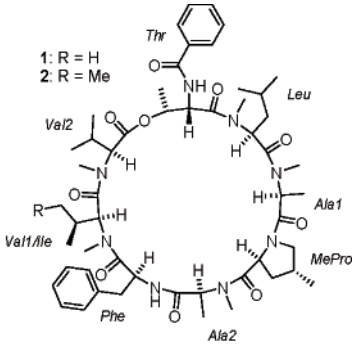

Four new cyclodepsipeptides, pteratides I−IV (**1**−**4**), have been isolated from the extract of a *Pterula* species collected from a Malaysian tropical forest. Homonuclear and heteronuclear 2D NMR techniques as well as MS fragmentation experiments, in combination with methanolysis, determined the gross structures of the peptides and showed that pteratides I and II each contained the nonproteinogenic amino acid 4-methylproline. The absolute configurations of the amino acids in pteratides I−IV were established using Marfey's method. Pteratides I and II are each potently cytotoxic against the P388 murine leukemia cell line (IC_{50} values of 41 and 40 nM, respectively). Pteratides III and IV show weaker, but still notable, activity with IC_{50} values of 7.4 and 2.9 μM, respectively.

Introduction

Many natural cyclodepsipeptides exhibit potent biological activities, for example, the cytotoxic and antiviral sansalvamide,[1] the fusaricidins,[2,3] which are antimicrobial against Gram-positive bacteria, and the antitumor agent kahalalide F,[4] which is currently in clinical trials against prostate cancer. We now report the isolation of a new group of highly cytotoxic cyclodepsipeptides, the pteratides, from the fruiting bodies of the coral-shaped Malaysian basidiomycete, identified as a *Pterula* species. Despite the wide geographical distribution, the secondary metabolites of this genus have not been extensively studied. The *Pterula* sp. most studied to date has been a cultivated strain, *Pterula* sp. 82168, which was found to produce the five previously unknown bioactive natural products hydroxystrobilurin A,[5] pterulones A and B,[6,7] pterulinic acid,[6] and noroudemansin A.[8] The *Pterula* sp. described here has also been the source of the pterulamides, six highly *N*-methylated linear peptides with unusual N- and C-end groups.[9]

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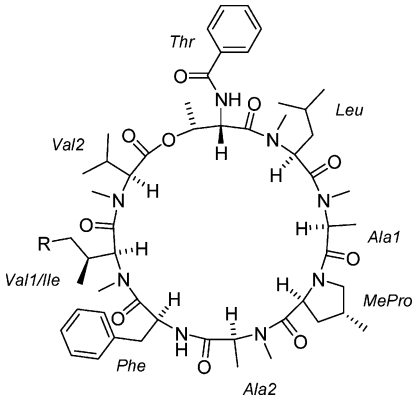
Results and Discussion

The MeOH extracts of three separate *Pterula* fruiting bodies (A−C) each exhibited potent cytotoxicity against P388 murine leukaemia cells (IC_{50} < 1 μg/mL). Despite morphological similarity, the extracts from the three fruiting bodies differed in their chemical profile as assessed by HPLC−UV−MS.[10] Bioactivity-guided isolation of the cytotoxic principles yielded four new cyclodepsipeptides: pteratides I (**1**; from A and C), II and III (**2** and **3**; from B), and IV (**4**; from C), along with the less cytotoxic linear pterulamides.[9] None of the previously described *Pterula* metabolites were found in these extracts. [5−8]



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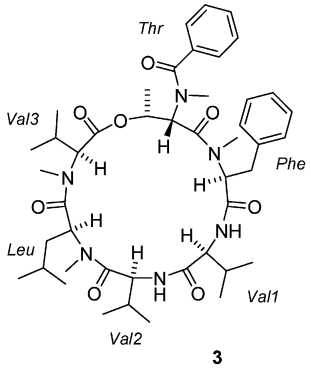
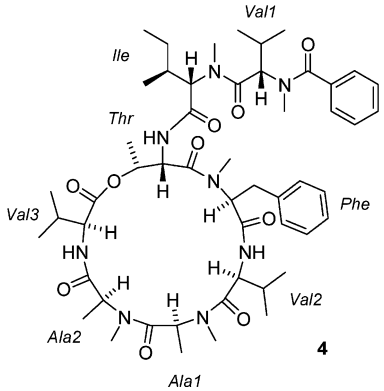


3



4

HRESIMS on pteratide I (**1**) established the mass of the pseudomolecular ion $[M + H]^+$ as 987.5918 Da, which corresponded to a molecular formula for **1** of $C_{53}H_{78}N_8O_{10}$. The ^{1}H NMR spectrum of **1** clearly revealed its peptidic nature; two amide resonances (δ 9.31 and δ 7.24), signals for eight α-protons in the range between 4.6 and 5.5 ppm, and five *N*-methyl groups between 2.5 and 3.2 ppm were observed. TOCSY, HSQC, and HMBC spectra were used to identify the constituent amino acids in **1**. Two valine, two alanine, one leucine, one phenylalanine, and one threonine unit could be readily identified. The signals of an additional aromatic group were attributed to a benzoyl residue.

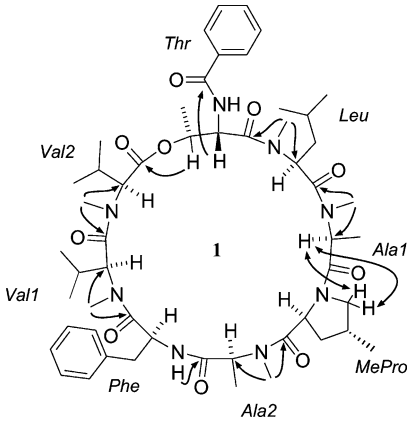


FIGURE 1. HMBC (single arrows) and NOESY (double arrows) correlations establishing the amino acid sequence of **1**.

The amino acid with the α-proton at δ_H 4.90 did not belong to the set of standard proteinogenic amino acids. In addition to this α-proton, there were also signals for two sets of diastereotopic methylene groups (δ_H 1.79 and 2.06, 2.93 and 3.89), a methine proton (δ_H 2.75), and a methyl group (δ_H 1.03). COSY, HMBC correlations and chemical shift arguments established the relationships between these groups leading to the identification of the amino acid as 4-methylproline (4-MePro). A ROESY correlation between the H-2 and the 4-methyl group and the absence of such a correlation between the H-2 and H-4 established a *trans*-relationship, i.e., (2*S**,4*R**)-configuration. The locations of the five *N*-methyl groups were determined using HMBC correlations to the α-carbons of the amino acid residues. By this approach the two valine, the two alanine, and the leucine residues were each found to be *N*-methylated. Long-range H,C-couplings from the amide proton at δ_H 7.24 were observed to the carbonyl carbons of the benzoyl group and to the threonine residue indicating *N*-benzoylation of this amino acid. HMBC correlations between the *N*-methyl groups, or the amide protons to the carbonyl carbon of the next respective amino acid, provided the necessary information for establishing the amino acid sequence of **1**. Two partial sequences were identified: NBz-Thr-NMe-Leu-NMe-Ala1 and 4-MePro-NMe-Ala2-NH-Phe-NMe-Val1-NMe-Val2 (Figure 1). An ester linkage between the β-hydroxyl group of the threonine residue and Val2 was evident from the long-range coupling of the threonine β-proton (δ_H 5.12) to the carbonyl carbon of Val2 (δ_C 168.7) and was supported by the low-field chemical shift of this β-proton. The remaining ring-closing connection between Ala1 and the 4-methylproline was confirmed by NOESY correlations between the α-proton of Ala1 and H-5a as well as H-5b of 4-MePro (Figure 1).

To confirm this sequence, pteratide I (**1**) was ring-opened by methanolysis, and the resultant methyl ester was analyzed by ESI MS/MS. The fragmentation of the sodiated molecular ion of the methyl ester of **1** (*m*/*z* 1041.7) gave y_5, y_6 and y_7

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fragment ions due to sequential losses of NBz-Thr, NMe-Leu, and NMe-Ala, respectively (see the Supporting Information). The a_6 and b_6 fragment ions were also observed in the MS/MS spectrum of **1** methyl ester confirming the presence of the fragment NBz-Thr-NMe-Leu-NMe-Ala-MePro-NMe-Ala-Phe.

The HRESIMS of pteratide II (**2**) showed the pseudomolecular ion $[M + Na]^+$ at *m*/*z* 1023.5902, indicating a molecular formula of $C_{54}H_{80}N_8O_{10}$ for this compound. The structural similarity to pteratide I (**1**) was immediately apparent from the ^{1}H NMR spectrum. Detailed analysis of the 2D NMR spectra revealed that the constituent amino acids were one valine, two alanine, one leucine, one isoleucine, one phenylalanine, and one *N*-benzoylated threonine. In addition to these amino acids, a *trans*-4-methylproline unit was again identified. Using the correlation data from the HMBC NMR experiment, it was found that the five *N*-methyl groups observable in the ^{1}H NMR spectrum were associated with the two alanine, valine, leucine, and the isoleucine residue. The sequence was established using HMBC correlations as NBz-Thr-NMe-Leu-NMe-Ala1-4-MePro-NMe-Ala2-NH-Phe-NMe-Ile-NMe-Val. As in **1**, the ring-closing ester bond was evident from the long-range coupling between the β-proton of threonine (δ_H 5.20) and the carbonyl carbon of the *C*-terminal valine (δ_C 168.8). Pteratide II (**2**) therefore is a homologue of pteratide I (**1**), with a valine substituted by isoleucine. The suggested sequence was again confirmed by ESI MS/MS analysis of the ring-opened methanolysis product of **2**.

Pteratide III (**3**), isolated from the same extract as **2**, had a pseudomolecular ion $[M + H]^+$ at *m*/*z* 819.5000, corresponding to a molecular formula for **3** of $C_{45}H_{66}N_6O_8$. The ^{1}H NMR spectrum again suggested a peptidic nature for this compound. As for the spectra of **1** and **2**, signals for amide protons, aromatic protons, α-protons of amino acids, *N*-methyl groups, and several overlapping methyl signals were observed. Analysis of the separate spin systems revealed that **3** contained six amino acid residues: three valine units, one leucine, one phenylalanine, and one threonine. The phenylalanine accounted for only half of the aromatic protons observed. The other aromatic protons belonged to a benzoyl group that was found to be attached to the amino group of threonine, as shown by a HMBC correlation of the α-proton of threonine (δ_H 5.74) to the carbonyl carbon of the benzoyl group (δ_C 174.4). The four *N*-methyl groups were attached to valine-3, leucine, phenylalanine, and threonine, which was thus both *N*-benzoylated and *N*-methylated. The sequence of the amino acids (HMBC correlation data) was NBz-NMe-Thr-NMe-Phe-NH-Val1-NH-Val2-NMe-Leu-NMe-Val3. Again, a long-range H,C-coupling from the β-proton of threonine (δ_H 5.68) to the carbonyl carbon of the *C*-terminal valine unit (δ_C 170.2) indicated the ring-closing ester bond between these two amino acids.

The molecular formula of pteratide IV (**4**) was found to be $C_{52}H_{78}N_8O_{10}$ by evaluation of HRESIMS and ^{13}C NMR data. Using TOCSY, HSQC, and HMBC data, this peptide was found to contain eight amino acids, five of them *N*-methylated, and also one benzoyl group. The sequence of these amino acids was elucidated using long-range H,C-couplings detected with the IMPRESS NMR technique, which provides higher resolution in the F1 dimension.[11] The sequence was determined as NBz-NMe-Val1-NMe-Ile-NH-Thr-NMe-Phe-NH-Val2-NMe-Ala1-NMe-Ala2-NH-Val3 with a ring-closing ester bond between Val3 and Thr.

(11) Crouch, R.; Boyer, R. D.; Johnson, R.; Krishnamurthy, K. *Magn. Reson. Chem.* **2004**, *42*, 301−307.

Using Marfey's method,[12] all amino acids in **1**−**4** were found to be of (*S*)-configuration. By comparison against standards the isoleucines found in **2** and **4**, the threonines in **1**, **2**, and **4** and the *N*-methylthreonine in **3** could be assigned as (2*S*,3*S*), (2*S*,3*R*), and (2*S*,3*R*), respectively. The 4-methylproline[13,14] in **1** and **2** was found in both cases to be (2*S*,4*R*).

Pteratides I (**1**) and II (**2**) exhibited potent cytotoxicity against P388 murine leukaemia cells with IC_{50} values of 41 nM (0.039 μg/mL) and 40 nM (0.039 μg/mL), respectively. Pteratides III (**3**) and IV (**4**) were 2 orders of magnitude less active than **1** and **2** with IC_{50} values of 7.4 μM (6.1 μg/mL) and 2.9 μM (2.9 μg/mL), respectively.

The pteratides have intriguing structures characterized by the high degree of *N*-methylation and, in the case of pteratides I−III (**1**-**3**), by *N*-benzoylation of the ring-closing threonine residues. Additionally, the rare nonproteinogenic amino acid (2*S*,4*R*)-4-methylproline is found in pteratides I and II (**1**, **2**). The (2*S*,4*S*) diastereoisomer of this unusual amino acid is known from the peptides of cyanobacteria,[15,16] while 4-methylproline from cyclopeptides of the sponge *Theonella* sp.[17] and an imperfect fungus[18] have undetermined stereochemistry. Beyond doubt, the most remarkable feature of pteratides is their potent cytotoxicity: the mechanism of action remains to be examined.

Experimental Section

Fungal Material. Fruiting bodies of an unidentified *Pterula* species were collected in the Sungkai Wildlife Forest, Perak, Malaysia (collections A and B) and at Krau, Pahang, Malaysia (collection C). Voucher specimens have been deposited in the collection at the School of Chemical Sciences and Food Technology, Universiti Kebangsaan Malaysia (collection A, UKM-F3384A; collection B, UKM-F3384B; collection C, UKM-F4794). Identification of the fungal material was made by one of the authors (A.L.J.C.) based on the characteristic ageotropic, multifid, brown fruit bodies growing on dead sticks. Fungal thalli from the three separate collections were cut into pieces and separately extracted with MeOH.

Isolation of Pteratides I−IV (1−4). The compounds were isolated by standard chromatographic methods (see the Supporting Information).

Pteratide I (1): amorphous white solid; $[\alpha]^{20}_D$ −128 (*c* 0.1, MeOH); for ^{1}H and ^{13}C NMR data see Table 1, for TOCSY, HMBC, and NOESY data see the Supporting Information; HRESIMS *m*/*z* 987.5918 $[M + H]^+$ (calcd for $C_{53}H_{79}N_8O_{10}$, 987.5919).

Pteratide II (2): amorphous white solid; $[\alpha]^{20}_D$ −40 (*c* 0.1, MeOH); for ^{1}H and ^{13}C NMR data see Table 1, for TOCSY, HMBC, and NOESY data see the Supporting Information; HRESIMS *m*/*z* 1023.5902 $[M + Na]^+$ (calcd for $C_{54}H_{80}N_8O_{10}Na$, 1023.5895).

Pteratide III (3): amorphous white solid; $[\alpha]^{20}_D$ −47 (*c* 0.1, MeOH); for ^{1}H and ^{13}C NMR data see Table 2, for TOCSY,

(12) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591−596.

(13) Golakoti, T.; Yoshida, W. Y.; Chaganty S.; Moore, R. E. *Tetrahedron* **2000**, *56*, 9093−9102.

(14) The four 4-MePro isomers were prepared by stereochemically selective routes. A paper describing the synthesis and the physical data for the stereoisomers and the Marfey derivatives is currently in preparation.

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TABLE 1. NMR Data for Pteratide I (1) and II (2)

		1[a]		**2**[b]				**1**[a]		**2**[b]	
position		^{13}C	^{1}H	^{13}C	^{1}H	position		^{13}C	^{1}H	^{13}C	^{1}H
Thr	NH		7.24		7.85	Ala2	NMe	29.0	2.50	28.2	2.32
	1	168.9		169.5			1	170.7		171.8	
	2	52.2	5.33	53.0	5.22		2	56.2	4.63	55.9	4.91
	3	73.0	5.12	72.5	5.20		3	15.0	2.50	13.5	1.22
	4	18.1	1.39	16.9	1.34	Phe	NH		9.31		9.31
	Bz-1	167.7		169.4			1	172.3		172.8	
	Bz-2	133.8		132.0			2	54.5	4.60	54.7	4.53
	Bz-3/7	127.5	7.85	127.1	7.77		3	35.0	3.05	34.0	3.00
	Bz-4/6	132.5	7.50	128.6	7.43				3.37		3.29
	Bz-5	129.1	7.58	132.1	7.51		4	139.0		138.5	
Leu	NMe	30.6	2.91	29.5	2.89		5/9	129.5	7.41	128.8	7.36
	1	169.2		169.7			6/8	128.7	7.26	128.2	7.16
	2	53.6	5.28	53.2	5.27		7	126.9	7.19	126.8	7.10
	3	37.5	1.34	37.0	1.29	Val1	NMe	29.9	3.24		
			1.97		1.83		1	171.1			
	4	25.0	1.50	24.7	1.43		2	57.7	5.10		
	5	22.5	0.91	21.4	0.83		3	27.6	2.45		
	6	23.2	0.93	21.9	0.84		4	18.5	0.92		
Ala1	NMe	29.3	2.58	29.0	2.54		5	20.2	0.88		
	1	168.6		168.4		Ile	NMe			29.2	3.14
	2	52.0	5.48	51.7	5.33		1			171.4	
	3	14.4	1.25	12.9	1.15		2			56.2	5.14
MePro	1	171.8		173.2			3			33.2	2.17
	2	56.4	4.90	55.7	5.24		3-Me			18.8	0.77
	3	36.0	1.79	35.3	1.81		4			23.9	0.93
			2.06		1.88						1.43
	4	33.5	2.75	33.2	2.53		5			15.0	0.78
	5	54.0	2.93	53.5	2.95	Val2	NMe	29.7	2.71	29.0	2.65
			3.89		3.72		1	168.7		168.8	
	4-Me	17.9	1.03	16.9	0.97		2	66.2	4.88	66.5	4.87
							3	27.8	2.31	27.0	2.42
							4	20.0	1.18	19.6	1.07
							5	19.9	0.98	19.1	0.88

[a] Recorded in $CDCl_3$ (500 MHz). [b] Recorded in MeOH-d_4 (500 MHz).

TABLE 2. NMR Data for Pteratide III (3) in MeOH-d_4

position		^{13}C[a]	^{1}H	position		^{13}C	^{1}H	position		^{13}C[a]	^{1}H
Thr	NMe	36.5	3.27	Phe	5/9	130.0	7.35	Leu	NMe	30.0	3.11
	1	172.7			6/8	128.9	7.31		1	174.1	
	2	61.8	5.74		7	127.2	7.25		2	50.5	5.48
	3	68.5	5.68	Val1	NH		7.66		3	37.1	1.34
	4	16.1	0.58		1	173.7					2.02
	Bz-1	174.4			2	54.1	4.51		4	25.1	1.59
	Bz-2	135.2			3	29.7	1.53		5	22.5	1.03
	Bz-3/7	128.3	7.47		4	16.1	0.16		6	21.3	1.07
	Bz-4/6	128.4	7.50		5	18.1	0.63	Val3	NMe	29.5	2.98
	Bz-5	131.2	7.50	Val2	NH		8.41		1	170.2	
Phe	NMe	30.7	3.04		1	174.2			2	65.4	4.39
	1	170.6			2	64.1	3.74		3	30.1	2.45
	2	63.3	5.05		3	29.5	2.29		4	19.7	1.05
	3	35.1	3.13		4	18.5	1.00		5	19.4	1.13
			3.68		5	19.4	1.09				
	4	137.8									

[a] The ^{13}C chemical shifts were determined from HSQC-DEPT and HMBC-CIGAR experiments.

HMBC, and NOESY data see the Supporting Information; HRES-IMS *m*/*z* 819.5000 $[M + H]^+$ (calcd for $C_{45}H_{67}N_6O_8$, 819.5020).

Pteratide IV (4): amorphous white solid; $[\alpha]^{20}_D$ −85 (*c* 0.33, MeOH); for ^{1}H and ^{13}C NMR data see Table 3, for TOCSY, HMBC, and NOESY data see the Supporting Information; HRES-IMS *m*/*z* 975.5940 $[M + H]^+$ (calcd for $C_{52}H_{79}N_8O_{10}$, 975.5919).

Methanolysis of Pteratides I and II (1, 2). The peptides **1** and **2** (20 μg each) were dissolved in dry MeOH (100 μL), and NaOMe solution (4%, 100 μL) was added. The mixtures were stirred for 3 h at 30 °C. After cooling, the solutions were evaporated to dryness and redissolved in ice H_2O−EtOAc (1:1) (1 mL). After solvent/solvent partitioning, the EtOAc layer for each compound was evaporated to dryness and analyzed by the ESI MS/MS.

Methyl Ester of 1: ESI MS/MS *m*/*z* 1041.7 (80) $[MCOOMe + Na]^+$, 997.8 (100) $[1041.7 - C_2H_4O]^+$, 836.8 (40) $[1041.7 - (NBz\text{-}Thr)]^+$, 783.5 (6) [(NBz-Thr-NMe-Leu-NMe-Ala-MePro-NMe-Ala-Phe) + $Na]^+$, 755.7 (20) $[783.5\text{-}CO]^+$, 709.7 (20) $[836.8 - (NMe\text{-}Leu)]^+$, 624.7 (10) $[709.7 - (NMeAla)]^+$.

Methyl Ester of 2: ESI MS/MS *m*/*z* 1055.7 (64) $[MCOOMe + Na]^+$, 1011.7 (100) $[1055.7 - C_2H_4O]^+$, 850.8 (12) [1055.7 −

TABLE 3. NMR Data for Pteratide IV (4) in $CDCl_3$

position		^{13}C	^{1}H	position		^{13}C	^{1}H	position		^{13}C	^{1}H
Val1	NMe	32.3	2.74	Thr	NH		7.15	Val2	3	29.7	2.26
	1	170.9			1	168.8			4	20.2	1.02
	2	58.6	5.28		2	54.85	4.46		5	15.8	0.66
	3	26.9	2.21		3	71.6	5.71	Ala1	NMe	31.3	3.27
	4	19.5	0.77		4	16.0	0.93		1	173.4	
	5	17.5	0.95	Phe	NMe	31.8	2.82		2	49.8	6.41
	Bz-1	171.5			1	168.52			3	15.0	1.29
	Bz-2	135.6			2	62.8	4.63	Ala2	NMe	28.7	2.72
	Bz-3/7	126.3	7.27		3	34.0	2.85		1	169.2	
	Bz-4/6	128.5	7.42				3.62		2	54.9	5.52
	Bz-5	129.9	7.33		4	136.9			3	14.7	1.36
Ile	NMe	30.3	3.09		5/9	128.8	7.28	Val3	NH		7.52
	1	169.6			6/8	128.7	7.30		1	168.46	
	2	60.4	4.45		7	127.1	7.30		2	58.2	4.57
	3	30.9	2.20	Val2	NH		7.15		3	32.3	1.89
	3-Me	16.1	0.70		1	172.6			4	18.1	0.77
	4	24.1	1.32		2	54.3	4.88		5	19.4	0.91
	5	9.8	0.84								

(NBz-Thr)]$^+$, 783.5 (6) [NBz-Thr-NMe-Leu-NMe-Ala-MePro-NMe-Ala-Phe + Na]$^+$, 755.7 (20) [783.5 − CO]$^+$, 723.7 (16) [850.8 − (NMeLeu)]$^+$, 638.7 (10) [723.7 − (NMeAla)]$^+$.

Stereochemical Analysis. The peptides **1**−**4** (0.5 mg each) were each hydrolyzed by heating (110 °C for 24 h) in HCl (6 M; 1 mL). After cooling, the solutions were evaporated to dryness and redissolved in H_2O (100 μL). A 1% (w/v) solution (100 μL) of FDAA (Marfey's reagent, N^{α}-(2,4-dinitro-5-fluorophenyl)-L-alaninamide)[12] in acetone was added to an aliquot (50 μL) of each acid hydrolysate solution (or to 50 μL of a 50 mM solution of the respective amino acid). After addition of $NaHCO_3$ solution (1 M; 20 μL), the mixture was incubated (1 h at 40 °C). The reaction was stopped by addition of HCl (2 M; 10 μL), the solvents were evaporated to dryness, and the residue was redissolved in MeOH−H_2O (1:1; 1 mL). An aliquot of each of these solutions (10 μL) was analyzed by HPLC (Prodigy C18, 250 × 4.6, 5 μm; solvents: A: water + 0.05% TFA, B: MeCN; linear gradient: 0 min 35% B, 30 min 45% B; 25 °C; 1 mL min^{-1}; detection at 330 nm). In all HPLC analyses of the FDAA derivatives the same column and flow rate were used and the compounds were detected at 330 nm. For the separation of the Thr, NMe-Thr, NMe-Ala, and 4-MePro FDAA derivatives, an alternative linear gradient was used (A: water + 0.05% TFA, B: MeOH; 0 min 45% B, 30 min 65% B). The FDAA derivatives of 4-MePro were analyzed by a third linear gradient (A: water + 0.05% HCOOH, B: MeOH; 0 min 45% B, 30 min 65% B). Retention times (min) of the FDAA amino acid derivatives used as standards together with the data for the observed peaks in the HPLC trace of the FDAA-derivatized hydrolysis products of **1**−**4** are included in the Supporting Information (Table T5).

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Supporting Information Available: General experimental procedures, 1D and 2D NMR spectra of pteratides (**1**−**4**), tables with 1D and 2D NMR data for **1**−**4**, and a table with data for HPLC analysis of the FDAA-derivatized hydrolysate of **1**−**4** and amino acid standards. This material is available free of charge via the Internet at http://pubs.acs.org.

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